Chapter 3. Results

In this chapter, we review the results of our literature search and summarize findings from studies that passed our screening and selection process. Studies examining the relationship between omega-3 fatty acids - eicosapentaenoic acid (EPA, 20:5 n-3), docosahexaenoic acid (DHA, 22:6 n-3), and alpha linolenic acid (ALA, 18:3 n-3) - and selected risk factors of cardiovascular disease (CVD) are summarized first, followed by studies that examine the correlation between omega-3 fatty acid intake and tissue levels of fatty acids.

Summary of Studies Found

Through the literature search we identified and screened over 7,464 abstracts indexed as English language articles concerning humans. We retrieved and screened 807 full text articles for potentially relevant human data. Of these, we rejected 463 articles for the reasons listed in the section "Listing of Excluded Studies" under "Rejected Studies". Of the remaining 344 articles, we analyzed risk factor and other outcome data from 123 (Table 3.1, "References and Included Studies" under "Included Studies"). The 221 non-rejected studies that were not analyzed are listed in the section "Listing of Excluded Studies" under "Studies Not Analyzed Because of Non-Randomized Design or Small Size". For most outcomes, we analyzed only the approximately 20 to 30 largest randomized trials. These trials were selected based on criteria described both in Table 3.1 and in the sections describing each risk factor included in this chapter.

We compiled an Evidence Table that provides detailed information about each study we analyzed (Appendix C, available electronically at http://www.ahrq.gov/clinic/epcindex.htm). The summary tables present specific information about each of the studies that we analyzed for a given risk factor or outcome. Information presented in the summary tables include: study design and size, amount of omega-3 fatty acid consumption, baseline level of the relevant risk factor, net change of risk factor level (change in omega-3 fatty acid arm less change in control arm), reported statistical significance of the net change, study quality, study population, and applicability for each study.

Most studies that we analyzed evaluated fish or other marine oils (as supplements, dietary fish, or oil spreads); few evaluated plant oils (as supplements, dietary oils, or oil spreads). Furthermore, few studies compared doses of similar omega-3 fatty acids, compared different omega-3 fatty acids, reported on potential covariates such as age and sex, analyzed effects based on duration of intake, or repeated measurements after subjects had stopped omega-3 fatty acid supplementation. Only 13 articles (reporting on 12 trials) reported any data related to either baseline dietary or experimental dietary intake of both omega-3 fatty acid and omega-6 fatty acid intake to allow an estimate of mean daily omega-6 to omega-3 fatty acid ratio [46-58]. However, no study analyzed the relationship between evaluated outcomes and either omega-6 to omega-3 fatty acid consumption ratio or combined omega-6 and omega-3 fatty acid consumption amounts. Any available data relating to relative amounts of omega-6 fatty acid consumption could not be evaluated separately from different doses or types of omega-3 fatty acids.

Note: Appendixes and Evidence Tables cited in this report are provided electronically at http://www.ahrq.gov/clinic/epcindex.htm

Each risk factor is discussed separately in the following, largely arbitrary, order:

- Lipids (total cholesterol, low density lipoprotein [LDL], high density lipoprotein [HDL], triglycerides, lipoprotein (a) [Lp(a)], apolipoproteins [apo] AI, B, B-100, and LDL apo B)

- Blood pressure

- Measures of glucose metabolism (hemoglobin A_{1c} [Hgb A_{1c}], fasting blood sugar [FBS], and fasting insulin)

- C-reactive protein (CRP)

- Measures of hemostasis (fibrinogen, factors VII and VIII, von Willebrand factor [vWF], and platelet aggregation)

- Non-serum diagnostic tests (coronary artery restenosis [following angioplasty], carotid intima-media thickness [IMT], exercise tolerance testing [ETT], and heart rate variability).

The final section of this chapter summarizes studies that examine the correlation between omega-3 fatty acid intake and tissue levels, including plasma or serum phospholipid levels, platelet phospholipids, erythrocyte membrane phospholipids, granulocyte membrane phospholipids, and monocyte membrane phospholipids.

Table 3.1 Numbers of studies of omega-3 fatty acids and cardiovascular risk factors

CVD Risk Factor	Total Studies Meeting Minimum Eligibility Criteria	Total Randomized Studies	Eligibility Criteria for Analysis [a]		Analyzed Studies
Lipids	182 [b]	108	RCT ≥ 60	Xover ≥ 40	**25**
Total Cholesterol	169	98	RCT ≥ 60	Xover ≥ 40	**23**
Low Density Lipoprotein	119	70	RCT ≥ 60	Xover ≥ 40	**15**
High Density Lipoprotein	141	81	RCT ≥ 60	Xover ≥ 40	**19**
Triglycerides	164	100	RCT ≥ 60	Xover ≥ 40	**19**
Lipoprotein (a)	23	14	RCT ≥ 5	Xover ≥ 5	**14**
Apolipoprotein A-1	61	37	RCT ≥ 20	Xover ≥ 15	**27**
Apolipoprotein B	52	29	RCT ≥ 20	Xover ≥ 10	**25**
Apolipoprotein B-100	11	10	RCT ≥ 5	Xover ≥ 5	**10**
Blood pressure	103	71	RCT ≥ 15 DM	Xover ≥ 10 DM	**6** [c]
Hemoglobin A_{1c}	32	22	RCT ≥ 10	Xover ≥ 10	**18**
Blood sugar, fasting	57	34	RCT ≥ 25	Xover ≥ 15	**17**
Fasting insulin	21	15	RCT ≥ 5	Xover ≥ 5	**15**
C-reactive protein	5	4	All		**5**
Fibrinogen	59	34	RCT ≥ 15	Xover ≥ 10	**24**
Factor VII	40	25	RCT ≥ 15	Xover ≥ 10	**19**
Factor VIII	13	5	RCT ≥ 5	Xover ≥ 5	**5**
von Willebrand factor	20	9	RCT ≥ 5	Xover ≥ 5	**9**
Platelet aggregation	84	39	RCT ≥ 15	Xover ≥ 10	**11** [d]
Coronary arteriography	17	14	RCT ≥ 5	Xover ≥ 5	**12** [e]
Carotid intima-media thickness	4	1	All		**4**
Exercise tolerance test	6	3	All		**6**
Heart rate variability	3	2	All		**3**
Sub-Total [f]	327	197			**123**
Risk Factors Not Analyzed					
Apolipoprotein C-III	3	1			
Remnant-like particles	2	0			
Free fatty acids or Non-esterified fatty acids	7	5			
Diabetes incidence	1	0			
Microalbuminuria	4	3			
Homocysteine	4	2			
Factor XII	4	1			
Bleeding time	48	21			
Interleukin 6	2	1			
VCAM-1 [g]	2	1			
Creatine kinase	5	4			
Echocardiography	1	1			
Endothelial function	11	8			
ECG parameters	4	3			
Heart rate, resting	23	16			
Ankle brachial index	1	1			
Total (Analyzed and not analyzed)	346				

a RCT ≥, minimum number of subjects in a parallel randomized controlled trial; Xover ≥, minimum number of subjects in a cross-over study; DM = diabetes mellitus.
b Minimum of 20 subjects consuming omega-3 fatty acids.
c We analyzed only studies of diabetic patients.
d We analyzed only studies with platelet aggregation data reported in text or table. We did not analyze studies that reported outcomes only in figures.
e We analyzed only studies that reported the number (or percent) of patients who had restenosis.
f Individual study numbers do not add up to totals because many articles reported more than 1 outcome.
g Vascular cell adhesion molecule 1

Lipids: Total Cholesterol

(Table 3.2)

Abnormal levels of serum lipids, primarily low density lipoprotein (LDL), high density lipoprotein (HDL), and triglycerides (Tg) have long been recognized as risk factors for CVD. Of interest is whether consuming omega-3 fatty acids as part of a therapeutic lifestyle change would improve lipid levels, or at least would not be detrimental. Recent National Cholesterol Education Program (NCEP) guidelines recommend a goal for fasting total cholesterol of less than 200 mg/dL in all adults, with lower levels recommended for people at elevated risk for CVD, including diabetics, smokers, people with hypertension or a family history of premature CVD, or who are beyond middle age [59].

Lipid levels are the most commonly measured CVD risk factor in trials of omega-3 fatty acid consumption. We found 182 studies that met eligibility criteria and reported data on the effect of omega-3 fatty acids on lipid levels in at least 20 subjects (See Table 3.1). Of these, we analyzed the 25 randomized trials with lipid data for at least 60 subjects in parallel trials and 40 subjects in crossover trials who consumed omega-3 fatty acids. It is important to note that because we analyzed only the largest randomized trials, we did not capture many smaller studies of diabetic patients.

Among these studies, 169 reported data on total cholesterol levels. We analyzed the 23 largest randomized trials.

Table 3.2 Effects of omega-3 fatty acids on total cholesterol (mg/dL) in randomized trials (6 weeks to 2 years)

Author, Year	Omega-3 Fatty Acid Arm [a]				Control		Results [b]			Quality [c]				
	N	Source		g/d	N	Source	Base	Net Δ	*P*	Summary	Jadad	Allocation Conceal	Applicability [d]	
		DHA/EPA Oils												
Cairns, 1996	325	Fish oil	ED	5.4	328	Corn oil	227	-3	NS	B	3	Un	CVD	II
Bonaa, 1992	71	Fish oil	ED	5.1	74	Corn oil	251	+2	NS	B	4	Un	DysLip	I
Lungershausen, 1994	42 [e]	Fish oil	ED	4.9	42 [e]	Corn oil	221	+2	NS	B	3	Un	CVD	II
Bairati, 1992b	66	Fish oil	ED	4.5	59	Olive oil	240	-1	NS	B	5	Un	CVD	II
Grimsgaard, 1997	75	Purified EPA	E	3.8	77	Corn oil	231	-10	.004 [f]	A	5	Un	GEN	I
	72	Purified DHA	D	3.7			232	-3	NS [f]					
Nilsen, 2001 [g]	75	Fish oil	ED	3.4	75	Corn oil	214	+9	NS	B	3	Un	CVD	II
Eritsland, 1995b	260	Fish oil	ED	3.3	251	No oil	252	-2	NS	B	2	Ad	CVD	II
Brox, 2001 [h]	38	Cod liver oil	ED	3.3	37	No oil	319	-19	NS	C	1	Un	DysLip	I
	37	Seal oil	ED	2.6			308	0	NS					
Franzen, 1993	92 [i]	Fish oil	ED	3.1	83 [i]	Olive	219	+2	nd	C	5	Ad	CVD	II
Osterud, 1995	26	Cod liver oil	ED	3.1	28	No oil	203	+1	NS	B	2	Un	GEN	I
	27	Seal/Cod oil	ED	2.8			204	+9	NS					
	27	Seal oil	ED	2.4			199	+2	NS					
	26	Whale oil	ED	1.7			197	+10	NS					
Leigh-Firbank, 2002	55 [e]	Fish oil	ED	3.0	55 [e]	Olive oil	255	-2	NS	B	3	Un	DysLip	I
Sacks, 1994	60 [j]	Fish oil	ED	2.4	66 [j]	Olive oil	190	+4	NS	C	3	Un	GEN	I

Continued

Table 3.2 Effects of omega-3 fatty acids on total cholesterol (mg/dL) in randomized trials (continued)

Author, Year	Omega-3 Fatty Acid Arm [a]				Control		Results [b]			Quality [c]				
	N	Source		g/d	N	Source	Base	Net Δ	*P*	Summary	Jadad	Allocation Conceal	Applicability [d]	
		DHA/EPA Oils (continued)												
Sirtori, 1998	459	Fish oil	ED	1.7 [k]	450	Olive oil	234 [L]	-1 [L]	NS	B	4	Ad	CVD risk [m]	I
von Schacky, 1999	89 [n]	Fish oil	ED	1.7 [o]	86 [p]	Plant oil	237	+6	NS	C	5	Ad	CVD	II
GISSI, 1999	2836	Fish oil	ED	0.9	2828	No oil	210	+2	NS	B	3	Un	CVD	II
	2830	Fish oil [q]			2830	Vitamin E	211	+4						
Leng, 1998	37 [r]	Fish oil	ED	0.045 [s]	36 [t]	Sunflower oil	233	+2	NS	C	4	Ad	CVD	II
		Plant Oils												
Natvig, 1968	289 [u]	Linseed oil	A	~5	316 [u]	Sunflower oil	246	+1	NS	C	2	Un	GEN	III
	47 [v]				51 [v]		250	+5	NS				DM [w]	III
Borchgrevink, 1966	100	Linseed oil	A	~5	100	Corn oil	289	+13	nd	C	2	Un	CVD	III
		Fish and Mediterranean Diets												
Singh, 2002	499	Indo-Mediterranean	T	1.8	501	NCEP I [x]	221	-20	<.0001	C	2	Un	CVD risk [y]	III
Hanninen, 1989	19	Fish (3.8/week)	ED	0.9	18	0.4 Fish/week	176	-8	nd	B	2	Un	GEN	III
	22	Fish (2.3/week)	ED	0.5			157	+2	nd					
	21	Fish (1.5/week)	ED	0.4			158	-7	nd					
	20	Fish (0.9/week)	ED	0.2			170	+3	nd					
de Lorgeril, 1994	171 [z]	Mediterranean/ Canola margarine	A	0.8% Kcal	168 [aa]	Regular	240	-1	NS	C	2	Un	CVD	II
		Combinations												
Mori, 1994	17	Fish oil & Fish diet [bb]	ED	5.2	18	Olive/Palm/ Safflower 40% fat diet	235 [cc]	+7 [dd]	NS	B	2	Un	CVD	II
	16	Fish oil	ED	4.2				+19 [dd]	NS					
	17	Fish diet [bb] & Placebo oil	ED	3.0				+13 [dd]	NS					
	17	Fish oil	ED	2.1				+21 [dd]	<.05					
	18	Fish diet [bb] & Placebo oil	ED	3.0	17	Oil 30% fat		+1 [dd]	NS					
Finnegan, 2003	31	Fish oil margarine/ Fish oil	ED	1.7	30	Sunflower margarine	212	+14	NS	A	4	Un	DysLip	I
	30	Fish oil margarine	ED	0.8			211	+4	NS					
	30	Rapeseed/Linseed margarine	A	4.5			217	+2	NS					

nd = no data

a A = ALA; D = DHA; E = EPA; ED = EPA+DHA; T = Total omega-3 fatty acids.

b Base = baseline level in treatment arm; Net Δ = net difference in change in omega-3 fatty acids arm compared with the change in control arm, see Methods; *P* = *P* value of difference between treatment and control arms; NS = not statistically significant.

c A = good quality; B = fair quality; C = poor quality; Jadad = Jadad Score (0-5, based on randomization, double blinding, and dropouts); Ad = adequate allocation concealment; In = inadequate allocation concealment; Un = allocation concealment unclear. See Methods.

d CVD = history of cardiovascular disease; DM I/II = diabetes mellitus type 1 or 2; DysLip = dyslipidemia; GEN = general, healthy population; N/IDDM = (non-) insulin dependent diabetes mellitus. I = broadly applicable; II = applicable to sub-group; III = narrow applicability. See Methods.

e Cross-over study.

f *P*=.04 for difference in effect of EPA and DHA.

g Only subjects who did not change statin treatment are included here.
h Data missing from article provided by study author.
i Maximum. Total analyzed was 172, not 175 (92+83).
j 84 at baseline.
k 2.6 g/day for first 2 months, then 1.7 g/day for 4 months.
L Estimate from graph.
m Dyslipidemia and one or more of: hypertension, diabetes, or glucose intolerance.
n 111 at baseline.
o 3.4 g/day for first 3 months, then 1.7 g/day for 21 months.
p 112 at baseline.
q Plus vitamin E 300 mg.
r Baseline data based on N=52.
s Plus 280 mg gamma linolenic acid (omega-6 fatty acid).
t Baseline data based on N=50.
u 33 missing data for one or both tests.
v 7 missing data for one or both tests.
w Sub-analysis.
x National Cholesterol Education Program step I prudent diet.
y One or more of: hypercholesterolemia, hypertension, diabetes, angina pectoris or myocardial infarction.
z Baseline data based on 289 subjects.
aa Baseline data based on 295 subjects.
bb Omega-3 fatty acid from fish diet is approximate, assuming that each of 4 different fish was consumed equally.
cc Mean baseline value for all subjects combined.
dd Estimated from graph.

Overall Effect [48,49,52,53,60-78]

Across the 23 studies there was a wide range of effects of omega-3 fatty acids on total cholesterol, although in most studies the net effect was small and generally of an increase in total cholesterol. Most studies found net increases of between 0% and 6% (approximately 0 to 14 mg/dL). Only 3 studies found that the changes in total cholesterol in subjects on omega-3 fatty acids were significantly different than control. Notably, the directions of the treatment effects were not consistent across these studies.

Sub-populations

Only 5 of the studies included generally healthy subjects, 3 of which were all male[66,67,72]. Net effects were generally small but inconsistent in direction. Most of the studies included subjects with a variety of types of CVD. There was no clear consistent effect among the 12 studies. Two studies evaluated subjects at increased risk of CVD with different sets of treatments and came to different conclusions. Sirtori et al. found no effect with fish oil in approximately 900 individuals with dyslipidemia and either hypertension, diabetes or glucose intolerance [77]. Singh et al. reported a large, highly significant reduction in total cholesterol with an Indo-Mediterranean diet in approximately 1,000 people with either hypercholesterolemia, hypertension, diabetes, angina or myocardial infarction [76]. However, this study found that subjects on the Indo-Mediterranean diet lost significantly more weight (3 kg) than those on the control diet. In addition, they reported uniform highly significant effects on all serum markers despite widely ranging effects. A number of statistical calculation errors were also found.

While no study evaluated a population of all diabetic subjects, Natvig et al., in an early Norwegian trial of linseed oil supplements, reported a sub-analysis of the 98 diabetic subjects and found that the effect of linseed oil was similar in both all subjects and specifically in diabetic

subjects, but that total cholesterol decreased by a small amount more in the diabetic subjects [72]. The difference was not significant.

Covariates

No subgroup analyses based on covariates were reported. Two studies performed regressions. Bairati et al. reported no change in total cholesterol effect after adjusting for age, sex, baseline lipid level, lipid treatment, body mass index and alcohol use [60]. Mori et al. performed a regression adjusting for change in weight and found a highly significant "group effect" increase in total cholesterol with omega-3 fatty acids ($P < .001$) [71]. This study also found larger relative net increases in total cholesterol among subjects on a 40% fat diet, but no net effect (and a decrease in absolute change) in subjects on a 30% fat diet. No clear difference was seen between the 5 studies that included only men and the remaining studies [61,66,67,71,72].

Dose and Source Effect

Three studies compared different sources – and doses – of marine oil supplements [62,66,74]. Grimsgaard et al. found a significantly greater decrease in total cholesterol with purified EPA than DHA in healthy, middle-aged men [66]. Brox et al. found a substantially greater decrease in total cholesterol with higher omega-3 fatty acid dose cod liver oil supplement than seal oil supplement in healthy subjects with elevated total cholesterol; although they imply that the difference was not statistically significant [62]. Osterud et al. found varying degrees of net increases of total cholesterol with different marine oil supplements in healthy subjects [74]. No clear pattern was evident among different doses of omega-3 fatty acids and dose effect of marine oil supplements was evident across the studies.

Hanninen et al. compared 5 fish diets [67]. No significant effect on total cholesterol was seen with any diet and there was no dose effect based on frequency of fish consumption.

Among subjects on a higher fat diet, there was no clear difference in effect based on source of EPA+DHA among men studied by Mori et al. [71]. Despite an apparent larger net increase in total cholesterol among subjects consuming both fish oil margarine and fish oil supplements compared to those consuming only fish oil margarine or rapeseed and linseed margarine, Finnegan et al. found no differences in effect among the treatments [53].

The 4 studies of ALA all reported net increases in total cholesterol, but there was no apparent difference compared to fish and fish oil studies.

Exposure Duration

In 7 studies, total cholesterol levels varied by similar amounts in treatment and control arms at multiple time points [49,53,67,69,73,75,77]. No differences in effect were seen at times ranging from 5 weeks to 2 years. No effect across studies is evident based on duration of intervention or exposure.

Sustainment of Effect

No study reported data on an effect after ceasing omega-3 fatty acid treatment.

Lipids: Low Density Lipoprotein

(Table 3.3)

Among the lipids commonly measured, the level of low density lipoprotein (LDL) is generally of most concern when determining CVD risk and whether to initiate therapy. The NCEP guidelines note that the relationship between LDL levels and CVD risk is continuous over a broad range of LDL levels from low to high [59]. Recommended goals for LDL level depend on an individual's CVD risk factors. Risk factors include diabetes, smoking, hypertension, family history of premature CVD, and being beyond middle age. With no or one risk factor, LDL goal is less than 160 mg/dL; with 2 or more risk factors, LDL goal is less than 130 mg/dL. People who already have CVD or who have diabetes are recommended to achieve an LDL of less than 100 mg/dL. As with total cholesterol, of interest is whether consuming omega-3 fatty acids as part of a therapeutic lifestyle change would improve LDL levels, or at least would not be detrimental.

Of the 25 randomized trials with lipid data for at least 60 subjects in parallel trials and 40 subjects in crossover trials who consumed omega-3 fatty acids 15 reported data on LDL (See Table 3.1).

Overall Effect [48,49,52,53,60,63-66,68-71,76,79]

The effect of omega-3 fatty acid consumption was fairly uniform across studies. Most found a net increase in LDL with treatment, although the range of effects varied substantially. Most studies found net increases of LDL of 10 mg/dL or less, although the complete range of mean net effects was a decrease of 19 mg/dL to an increase of 21 mg/dL. As with a number of other outcomes, Singh et al. found a discordant result [76]. In this case, they reported a large, highly significant reduction in LDL with an Indo-Mediterranean diet in subjects at risk for CVD. However, as previously noted, this study found a difference in weight loss between the 2 interventions and reported uniform highly significant effects on all serum markers despite widely ranging effects; also, a number of statistical calculation errors were found.

Sub-populations

Only a single study included generally healthy subjects and no study included exclusively diabetics. Most of the studies included subjects with CVD. There was no clear difference among the 10 studies of CVD populations compared to the 3 dyslipidemia studies or single study of healthy subjects.

Covariates

No subgroup analyses based on covariates were reported. Two studies performed regressions. Bairati et al. reported that the effect of fish oil supplements on LDL (a net increase) was reduced and became borderline non-significant ($P = .06$) after adjusting for age, sex, baseline lipid level, lipid treatment, body mass index and alcohol use [60]. Mori et al. performed a regression adjusting for change in weight and found a highly significant "group effect" increase in LDL with omega-3 fatty acids ($P < .001$) [71]. In contrast to their findings for total cholesterol, they reported similar effects on LDL among subjects on a 40% fat diet and on a 30% fat diet.

Table 3.3 Effects of omega-3 fatty acids on low density lipoprotein (mg/dL) in randomized trials (6 weeks to 2 years)

Author, Year	Omega-3 Fatty Acid Arm [a]				Control		Results [b]			Quality [c]				
	N	Source		g/d	N	Source	Base	Net Δ	*P*	Summary	Jadad	Allocation Conceal	Applicability [d]	
		DHA/EPA Oils												
Cairns, 1996	325	Fish oil	ED	5.4	328	Corn oil	148	+3	NS	B	3	Un	CVD	II
Bonaa, 1992	70	Fish oil	ED	5.1	68	Corn oil	177	+7	NS	B	4	Un	DysLip	I
Lungers-hausen, 1994	42 [e]	Fish oil	ED	4.9	42 [e]	Corn oil	156	+7	NS	B	3	Un	CVD	II
Bairati, 1992b	66	Fish oil	ED	4.5	59	Olive oil	158	+12	<.05	B	5	Un	CVD	II
Grimsgaard, 1997	75	Purified EPA	E	3.8	77	Corn oil	157	-5	NS	A	5	Un	GEN	I
	72	Purified DHA	D	3.7			157	0	NS					
Eritsland, 1995b	260	Fish oil	ED	3.3	251	No oil	177	+4	NS	B	2	Ad	CVD	II
Franzen, 1993	92 [f]	Fish oil	ED	3.1	83 [f]	Olive	151	+9	nd	C	5	Ad	CVD	II
Leigh-Firbank, 2002	55 [e]	Fish oil	ED	3.0	55 [e]	Olive oil	175	+13	.03	B	3	Un	DysLip	I
Angerer, 2002	87	Fish oil	ED	1.7	84	Fatty acid	157	+6	NS	B	4	Ad	CVD	II
GISSI, 1999	2836	Fish oil	ED	0.9	2828	No oil	137	+3	NS	B	3	Un	CVD	II
	2830	Fish oil [g]			2830	Vitamin E	138	+5						
Leng, 1998	37 [h]	Fish oil	ED	0.045 [i]	36 [j]	Sunflower oil	107	+6	NS	C	4	Ad	CVD	II
		Fish and Mediterranean Diets												
Singh, 2002	499	Indo-Mediterranean	T	1.8	501	NCEP I [k]	141	-19	<.0001	C	2	Un	CVD risk [L]	III
de Lorgeril, 1994	171 [m]	Mediterranean/ Canola margarine	A	0.8% Kcal	168 [n]	Regular	175	+3	NS	C	2	Un	CVD	II
		Combinations												
Mori, 1994	17	Fish oil & Fish diet [o]	ED	5.2	18	Olive/Palm/ Safflower 40% fat diet	157 [p]	+11 [q]	NS	B	2	Un	CVD	II
	16	Fish oil	ED	4.2				+21 [q]	<.01					
	17	Fish diet [o] & Placebo oil	ED	3.0				+10 [q]	NS					
	17	Fish oil	ED	2.1				+16 [q]	<.05					
	18	Fish diet [o] & Placebo oil	ED	3.0	17	Oil 30% fat		+12 [q]	<.05					
Finnegan, 2003	31	Fish oil margarine/ Fish oil	ED	1.7	30	Sunflower margarine	132	+13	NS	A	4	Un	DysLip	I
	30	Fish oil margarine	ED	0.8			132	0	NS					
	30	Rapeseed/Linseed margarine	A	4.5			137	-2	NS					

nd = no data

a A = ALA; D = DHA; E = EPA; ED = EPA+DHA; T = Total omega-3 fatty acids.

b Base = baseline level in treatment arm; Net Δ = net difference in change in omega-3 fatty acids arm compared with the change in control arm, see Methods; *P* = *P* value of difference between treatment and control arms; NS = not statistically significant.

c A = good quality; B = fair quality; C = poor quality; Jadad = Jadad Score (0-5, based on randomization, double blinding, and dropouts); Ad = adequate allocation concealment; In = inadequate allocation concealment; Un = allocation concealment unclear. See Methods.

d CVD = history of cardiovascular disease; DM I/II = diabetes mellitus type 1 or 2; DysLip = dyslipidemia; GEN = general, healthy population; N/IDDM = (non-) insulin dependent diabetes mellitus. I = broadly applicable; II = applicable to sub-group; III = narrow applicability. See Methods.

e Cross-over study.

f Maximum. Total analyzed was 172, not 175 (92+83).

g Plus vitamin E 300 mg.
h Baseline data based on N=52.
i Plus 280 mg gamma linolenic acid (omega-6 fatty acid).
j Baseline data based on N=50.
k National Cholesterol Education Program step I prudent diet.
L One or more of: hypercholesterolemia, hypertension, diabetes, angina pectoris or myocardial infarction.
m Baseline data based on 289 subjects.
n Baseline data based on 295 subjects.
o Omega-3 fatty acid from fish diet is approximate, assuming that each of 4 different fish was consumed equally.
p Mean baseline value for all subjects combined.
q Estimated from graph.

Dose and Source Effect

Mori et al. found no difference in effect among men consuming various doses of EPA+DHA either as supplements or as dietary fish [71]. Finnegan et al. noted a particularly large increase in LDL in the fish oil margarine/fish oil supplement arm compared to other arms, but the differences were not statistically significant [53]. Grimsgaard found no difference in effect on LDL level between purified EPA and purified DHA [66].

The 2 studies of ALA reported smaller net changes in LDL, but it is not clear that this represents a real difference in effect.

Exposure Duration

In 3 studies, LDL levels varied by similar amounts in treatment and control arms at multiple time points [49,53,69]. No differences in effect were seen at times ranging from 8 weeks to 2 years. No effect across studies is evident based on duration of intervention or exposure.

Sustainment of Effect

No study reported data on an effect after ceasing omega-3 fatty acid treatment.

Lipids: High Density Lipoprotein

(Table 3.4)

High density lipoprotein (HDL) plays a primary function in removing lipids from the bloodstream to be processed in the liver. Therefore, people with reduced levels of HDL are at increased risk of CVD independent of LDL or Tg levels. The new NCEP guidelines categorize an HDL level of less than 40 mg/dL as low, implying an increased risk of CVD [59]. Commonly used and well-tolerated drugs for dyslipidemia generally have at most a modest effect on HDL levels. Lifestyle changes, including physical exercise and low saturated fat diets are generally recommended to help increase HDL. Of interest is whether consuming omega-3 fatty acids as part of a therapeutic lifestyle change would help improve HDL levels, or at least that it would not be detrimental.

Of the 25 randomized trials with lipid data for at least 60 subjects in parallel trials and 40 subjects in crossover trials who consumed omega-3 fatty acids 19 reported data on HDL (See Table 3.1).

Table 3.4 Effects of omega-3 fatty acids on high density lipoprotein (mg/dL) in randomized trials (6 weeks to 2 years)

Author, Year	Omega-3 Fatty Acid Arm [a]				Control		Results [b]			Quality [c]				
	N	Source		g/d	N	Source	Base	Net Δ	*P*	Summary	Jadad	Allocation Conceal	Applicability [d]	
		DHA/EPA Oils												
Cairns, 1996	325	Fish oil	ED	5.4	328	Corn oil	40	0	NS	B	3	Un	CVD	II
Bonaa, 1992	70	Fish oil	ED	5.1	69	Corn oil	51	-1	NS	B	4	Un	DysLip	I
Lungers-hausen, 1994	42 [e]	Fish oil	ED	4.9	42 [e]	Corn oil	40	+1	NS	B	3	Un	CVD	II
Grimsgaard, 1997	75	Purified EPA	E	3.8	77	Corn oil	51	+1	NS [f]	A	5	Un	GEN	I
	72	Purified DHA	D	3.7			53	+3	.0005 [f]					
Bairati, 1992b	66	Fish oil	ED	4.5	59	Olive oil	40	+4	<.05	B	5	Un	CVD	II
Nilsen, 2001 [g]	119	Fish oil	ED	3.4	120	Corn oil	42	+5	<.05	B	3	Un	CVD	II
Eritsland, 1995b	260	Fish oil	ED	3.3	251	No oil	41	+1	NS	B	2	Ad	CVD	II
Brox, 2001 [h]	38	Cod liver oil	ED	3.3	37	No oil	50	0	NS	C	1	Un	DysLip	I
	37	Seal oil	ED	2.6			50	+4	NS					
Franzen, 1993	92 [i]	Fish oil	ED	3.1	83 [i]	Olive	43	+2	nd	C	5	Ad	CVD	II
Osterud, 1995	26	Cod liver oil	ED	3.1	28	No oil	48	+3	NS	B	2	Un	GEN	I
	27	Seal/Cod oil	ED	2.8			53	+4	<.05					
	27	Seal oil	ED	2.4			51	+2	NS					
	26	Whale oil	ED	1.7			49	+5	<.005					
Leigh-Firbank, 2002	55 [e]	Fish oil	ED	3.0	55 [e]	Olive oil	37	0	NS	B	3	Un	DysLip	I
Sacks, 1994	60 [j]	Fish oil	ED	2.4	66 [j]	Olive oil	46	+2	NS	C	3	Un	GEN	I
Angerer, 2002	87	Fish oil	ED	1.7	84	Fatty acid	51	-3	NS	B	4	Ad	CVD	II
GISSI, 1999	2836	Fish oil	ED	0.9	2828	No oil	42	0	NS	B	3	Un	CVD	II
	2830	Fish oil [k]			2830	Vitamin E	42	0						
Leng, 1998	37 [L]	Fish oil	ED	0.045 [m]	36 [n]	Sunflower oil	45	+1	NS	C	4	Ad	CVD	II
		Fish and Mediterranean Diets												
Singh, 2002	499	Indo-Mediterranean	T	1.8	501	NCEP I [o]	45	+2	<.0001	C	2	Un	CVD risk [p]	III
de Lorgeril, 1994	171 [q]	Mediterranean/ Canola margarine	A	0.8% Kcal	168 [r]	Regular	45	-1	NS	C	2	Un	CVD	II
		Combinations												
Mori, 1994	17	Fish oil & Fish diet [s]	ED	5.2	18	Olive/Palm/ Safflower 40% fat diet	48 [t]	+3 [u]	<.01	B	2	Un	CVD	II
	16	Fish oil	ED	4.2				+2 [u]	<.05					
	17	Fish diet [s] & Placebo oil	ED	3.0				+3 [u]	<.001					
	17	Fish oil	ED	2.1				+4 [u]	<.01					
	18	Fish diet [s] & Placebo oil	ED	3.0	17	Oil 30% fat		+3 [u]	<.05					
Finnegan, 2003	31	Fish oil margarine/ Fish oil	ED	1.7	30	Sunflower margarine	52	+2	NS	A	4	Un	DysLip	I
	30	Fish oil margarine	ED	0.8			53	+3	NS					
	30	Rapeseed/Linseed margarine	A	4.5			50	+1	NS					

nd = no data

a A = ALA; D = DHA; E = EPA; ED = EPA+DHA; T = Total omega-3 fatty acids.

b Base = baseline level in treatment arm; Net Δ = net difference in change in omega-3 fatty acids arm compared with the change in control arm, see Methods; *P* = *P* value of difference between treatment and control arms; NS = not statistically significant.
c A = good quality; B = fair quality; C = poor quality; Jadad = Jadad Score (0-5, based on randomization, double blinding, and dropouts); Ad = adequate allocation concealment; In = inadequate allocation concealment; Un = allocation concealment unclear. See Methods.
d CVD = history of cardiovascular disease; DM I/II = diabetes mellitus type 1 or 2; DysLip = dyslipidemia; GEN = general, healthy population; N/IDDM = (non-) insulin dependent diabetes mellitus. I = broadly applicable; II = applicable to sub-group; III = narrow applicability. See Methods.
e Cross-over study.
f *P*=.009 for difference in effect of EPA and DHA.
g All subjects regardless of whether statin treatment changed during study.
h Data missing from article provided by study author.
i Maximum. Total analyzed was 172, not 175 (92+83).
j 84 at baseline.
k Plus vitamin E 300 mg.
L Baseline data based on N=52.
m Plus 280 mg gamma linolenic acid (omega-6 fatty acid).
n Baseline data based on N=50.
o National Cholesterol Education Program step I prudent diet.
p One or more of: hypercholesterolemia, hypertension, diabetes, angina pectoris or myocardial infarction.
q Baseline data based on 289 subjects.
r Baseline data based on 295 subjects.
s Omega-3 fatty acid from fish diet is approximate, assuming that each of 4 different fish was consumed equally.
t Mean baseline value for all subjects combined.
u Estimated from graph.

Overall Effect [48,49,52,53,60,62-66,68-71,73-76,79]

The effect of omega-3 fatty acid consumption was generally consistent across the 19 studies. Most found a small net increase in HDL with treatment of up to 3 to 5 mg/dL, although 7 found a small net decrease or no effect in at least one tested study arm. Six of the studies reported that the net increase in HDL was statistically significant.

Sub-populations

Across studies, there is no clear difference in effect among the 11 studies of CVD populations, the 4 studies of dyslipidemic patients, the 3 studies of healthy subjects, or the study of Indians at increased risk of CVD. No study included only diabetic patients.

Covariates

No subgroup analyses based on covariates were reported. Two studies performed regressions. Bairati et al. reported that the effect of fish oil supplements on HDL (a net increase) was reduced and became borderline non-significant ($P = .06$) after adjusting for age, sex, baseline lipid level, lipid treatment, body mass index and alcohol use [60]. Mori et al. performed a regression adjusting for change in weight and found a highly significant "group effect" increase in HDL with omega-3 fatty acids ($P < .001$) [71]. In contrast with their findings for total cholesterol, they reported similar effects on HDL among subjects on a 40% fat diet and those on a 30% fat diet.

Dose and Source Effect

Three studies compared different sources – and doses – of marine oil supplements [62,66,74]. Grimsgaard et al. found a small difference in effect between purified EPA and DHA, but the net increase in HDL was significantly larger in men consuming DHA than those consuming EPA [66]. In studies by Brox et al. and Osterud et al., somewhat different net effects were seen with the different types of oils; however, neither study reported on whether the oils differed from each other on their effect on HDL [62,74]. No dose effect of marine oil supplements was evident across the studies.

Mori et al. found no difference in effect among men consuming various doses of EPA+DHA either as supplements or as dietary fish [71]. All doses and sources of omega-3 fatty acids resulted in significant increases in HDL. Finnegan et al. reported no difference in effect with different omega-3 fatty acid treatments [53].

Only 2 studies tested ALA supplementation, with minimal effect.

Exposure Duration

Five studies reported data on time trends of HDL levels. Leng et al., de Lorgeril et al. and Finnegan et al. reported no difference in HDL levels at multiple time periods between 8 weeks and 2 years. [49,53,69]. In contrast, Nilsen et al. reported a steady increase in HDL in patients with recent myocardial infarctions who started fish oil supplements at 6 weeks (+8%), 6 months (+14%), and 12 months (+19%); patients on corn oil had variable HDL levels (-0.3%, +4%, and +7%, respectively). Sacks et al. reported that HDL levels were unchanged at 3 months in healthy subjects taking fish oil supplements compared to control – decreasing by about 1.5 mg/dL in both – but that HDL returned to baseline at 6 months, resulting in a small net difference compared to control. No clear effect across studies is evident based on duration of intervention or exposure.

Sustainment of Effect

No study reported data on an effect after ceasing omega-3 fatty acid treatment.

Lipids: Triglycerides

(Table 3.5, Figures 3.1 and 3.2)

Elevated levels of triglycerides (Tg) are increasingly being recognized as a risk factor for CVD, independent of other serum lipids. Elevated Tg are most frequently seen in patients with the metabolic syndrome, although various secondary and genetic factors can raise Tg. The recent NCEP guidelines recommend a goal for fasting Tg of less than 150 mg/dL [59]. Fish oil's ability to lower Tg is considered one of the leading mechanisms by which omega-3 fatty acid consumption lowers CVD risk [80].

Of the 25 randomized trials with lipid data for at least 60 subjects in parallel trials and 40 subjects in crossover trials who consumed omega-3 fatty acids 19 reported data on Tg (See Table 3.1).

Overall Effect [48,49,52,53,60,63-68,70,71,73,74,76,77,79,81]

With few exceptions, Tg levels in the 19 studies decreased by substantial amounts in subjects taking omega-3 fatty acids, both in absolute amount and compared to control groups. The changes in Tg were generally highly significant.

Table 3.5 Effects of omega-3 fatty acids on triglycerides (mg/dL) in randomized trials (6 weeks to 2 years)

Author, Year	Omega-3 Fatty Acid Arm [a]				Control		Results [b]			Quality [c]				
	N	Source		g/d	N	Source	Base	Net Δ	*P*	Summary	Jadad	Allocation Conceal	Applicability [d]	
		DHA/EPA Oils												
Cairns, 1996	325	Fish oil	ED	5.4	328	Corn oil	235	-64	<.05	B	3	Un	CVD	II
Bonaa, 1992	72	Fish oil	ED	5.1	72	Corn oil	124	-23	<.01	B	4	Un	DysLip	I
Lungershausen, 1994	42 [e]	Fish oil	ED	4.9	42 [e]	Corn oil	150	-19	<.01	B	3	Un	CVD	II
Bairati, 1992b	66	Fish oil	ED	4.5	59	Olive oil	204	-80	<.0001	B	5	Un	CVD	II
Grimsgaard, 1997	75	Purified EPA	E	3.8	77	Corn oil	109	-23	.0001 [f]	A	5	Un	GEN	I
	72	Purified DHA	D	3.7			110	-29	.0001 [f]					
Nilsen, 2001 [g]	61	Fish oil (men)	ED	3.4	61	Corn oil	140	-50	.001	B	3	Un	CVD	II
	12	Fish oil (women)			13		123	-71	.07					
Eritsland, 1995b	260	Fish oil	ED	3.3	251	No oil	172	-32	<.0001	B	2	Ad	CVD	II
Franzen, 1993	92 [h]	Fish oil	ED	3.1	83 [h]	Olive	158	-34	nd	C	5	Ad	CVD	II
Osterud, 1995	26	Cod liver oil	ED	3.1	28	No oil	113	-28	<.05	B	2	Un	GEN	I
	27	Seal/Cod oil	ED	2.8			114	-21	NS					
	27	Seal oil	ED	2.4			106	-14	NS					
	26	Whale oil	ED	1.7			97	-9	NS					
Leigh-Firbank, 2002	55 [e]	Fish oil	ED	3.0	55 [e]	Olive oil	221	-74	<.001	B	3	Un	DysLip	I
Maresta, 2002	125	Fish oil	ED	2.6 [i]	132	Olive oil	160	+5	NS	B	3	Un	CVD	II
Sirtori, 1998	459	Fish oil	ED	1.7 [j]	450	Olive oil	294 [k]	-63	<.0001	B	4	Ad	CVD risk [L]	I
Angerer, 2002	87	Fish oil	ED	1.7	84	Fatty acid	194	-22	NS	B	4	Ad	CVD	II
GISSI, 1999	2836	Fish oil	ED	0.9	2828	No oil	163	-8	<.05	B	3	Un	CVD	II
	2830	Fish oil [m]			2830	Vitamin E	160	-6						
		Fish and Mediterranean Diets												
Singh, 2002	499	Indo-Mediterranean	T	1.8	501	NCEP I [n]	163	-22	<.0001	C	2	Un	CVD risk [o]	III
Hanninen, 1989	19	Fish (3.8/week)	ED	0.9	18	0.4 Fish/week	81	-14	nd [p]	B	2	Un	GEN	III
	22	Fish (2.3/week)	ED	0.5			81	-12	nd [q]					
	21	Fish (1.5/week)	ED	0.4			60	-8	nd [q]					
	20	Fish (0.9/week)	ED	0.2			69	+4	NS					
de Lorgeril, 1994	171 [r]	Mediterranean/ Canola margarine	A	0.8% Kcal	168 [s]	Regular	190	-19	NS	C	2	Un	CVD	II
		Combinations												
Mori, 1994	17	Fish oil & Fish diet [t]	ED	5.2	18	Olive/Palm/ Safflower	154 [u]	-65 [k]	<.001	B	2	Un	CVD	II

Author, Year	Omega-3 Fatty Acid Arm [a]				Control		Results [b]			Quality [c]				Applicability [d]
	N	Source		g/d	N	Source	Base	Net Δ	*P*	Summary	Jadad	Allocation Conceal		
	16	Fish oil	ED	4.2		40% fat diet		-56 [k]	<.01					
	17	Fish diet [t] & Placebo oil	ED	3.0				-32 [k]	<.001					
	17	Fish oil	ED	2.1				-21 [k]	<.05					
	18	Fish diet [t] & Placebo oil	ED	3.0	17	Oil 30% fat		-37 [k]	<.01					
Finnegan, 2003	31	Fish oil margarine / Fish oil	ED	1.7	30	Sunflower margarine	142	-10	NS	A	4	Un	DysLip	I
	30	Fish oil margarine	ED	0.8			146	+6	NS					
	30	Rapeseed/Linseed margarine	A	4.5			147	+23	NS					

nd = no data

a A = ALA; D = DHA; E = EPA; ED = EPA+DHA; T = Total omega-3 fatty acids.

b Base = baseline level in treatment arm; Net Δ = net difference in change in omega-3 fatty acids arm compared with the change in control arm, see Methods; *P* = *P* value of difference between treatment and control arms; NS = not statistically significant.

c A = good quality; B = fair quality; C = poor quality; Jadad = Jadad Score (0-5, based on randomization, double blinding, and dropouts); Ad = adequate allocation concealment; In = inadequate allocation concealment; Un = allocation concealment unclear. See Methods.

d CVD = history of cardiovascular disease; DM I/II = diabetes mellitus type 1 or 2; DysLip = dyslipidemia; GEN = general, healthy population; N/IDDM = (non-) insulin dependent diabetes mellitus. I = broadly applicable; II = applicable to sub-group; III = narrow applicability. See Methods.

e Cross-over study.

f Non-significant difference in effect between EPA and DHA.

g Only subjects who did not change statin treatment are included here.

h Maximum. Total analyzed was 172, not 175 (92+83).

i 5.1 g for 1 month before and 1 month after PTCA, then reduced to 2.6 g for an additional 5 months.

j 2.6 g/day for first 2 months, then 1.7 g/day for 4 months.

k Estimate from graph.

L Dyslipidemia and one or more of: hypertension, diabetes, or glucose intolerance.

m Plus vitamin E 300 mg.

n National Cholesterol Education Program step I prudent diet.

o One or more of: hypercholesterolemia, hypertension, diabetes, angina pectoris or myocardial infarction.

p *P*<.02 change from baseline.

q *P*<.10 change from baseline.

r Baseline data based on 289 subjects.

s Baseline data based on 295 subjects.

t Omega-3 fatty acid from fish diet is approximate, assuming that each of 4 different fish was consumed equally.

u Mean baseline value for all subjects combined.

Sub-populations

The 3 studies of healthy subjects, whose mean Tg levels were normal, generally found net decreases in Tg levels of about 10% to 25%. Eleven studies included subjects with a variety of types of CVD, all with mean Tg levels above 150 mg/dL. With the exception of Maresta et al., the 11 studies reported net decreases in Tg of between about 10% to 30%, most of which were statistically significant [81]. There was no obvious difference between the study by Maresta et al. of patients undergoing PTCA and other studies to explain the discordant finding.

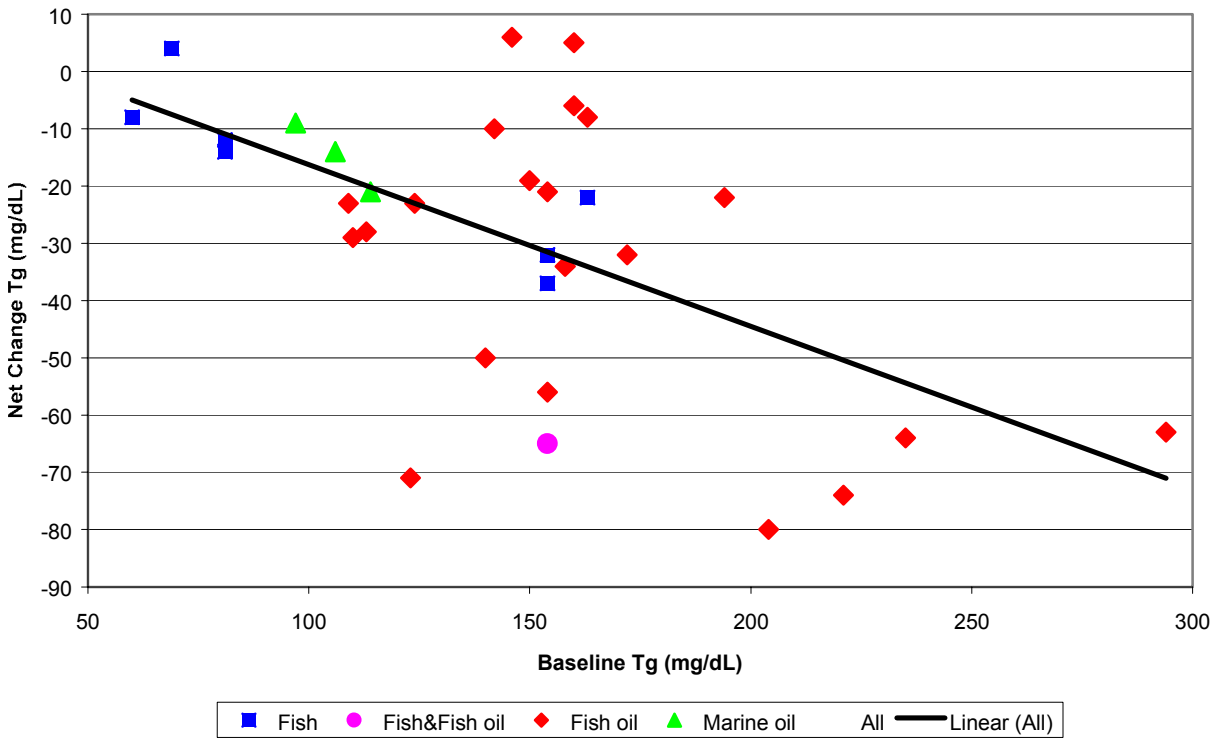
Two studies evaluated subjects at increased risk of CVD with different sets of treatments. Both of these studies found large, significant reductions in Tg. Two of 3 studies of dyslipidemic

patients reported large net decreases in Tg of 20% or 33%. Finnegan et al., in a study of moderately hyperlipidemic patients, found different effects of omega-3 fatty acid consumption on Tg depending on dose and source [53]. No study evaluated a population of only diabetic subjects.

Covariates

Nilsen et al. found similar decreases in Tg among men and women, where the difference in significance level can be ascribed mostly to sample size [73]. Two studies that performed regressions both found no substantial change in the significant Tg reduction after adjusting for age, sex, baseline lipid level, lipid treatment, body mass index and alcohol use [60] or change in weight [71]. Grimsgaard et al. reported the effect of purified EPA and DHA on Tg in quartiles of baseline Tg [66]. While the authors did not discuss whether the effect of omega-3 fatty acids was associated with baseline Tg level, there does appear to be a trend toward greater reduction of Tg in subjects with higher baseline Tg. Those in the lowest quartile had a net reduction of approximately 7 mg/dL (10 – 14%); those in the middle two quartiles had net reductions of between 15 mg/dL and 27 mg/dL (14 – 30%); and those in the highest quartile (128 mg/dL – 319 mg/dL) had net decreases in Tg of about 50 mg/dL (about 28%). Across studies, the average net decrease in Tg level was larger in studies with higher mean baseline levels, as indicated by Figure 3.1, in which the meta-regression is not adjusted for dose of omega-3 fatty acid or study size. After adjusting for dose and the study variance, the association across studies remains statistically significant. In a separate analysis comparing different percentages of fat in the diet, Mori et al. also found nearly identical effects in subjects on 30% or 40% fat diets who were consuming similar amounts of omega-3 fatty acids [71].

Figure 3.1 Meta-regression of baseline triglyceride (Tg) level versus net change in Tg. Each point represents an individual study or study arm. Marine oils include non-fish animal sources including Minke whale and seal. Regression not adjusted for dose of omega-3 fatty acid or study size.

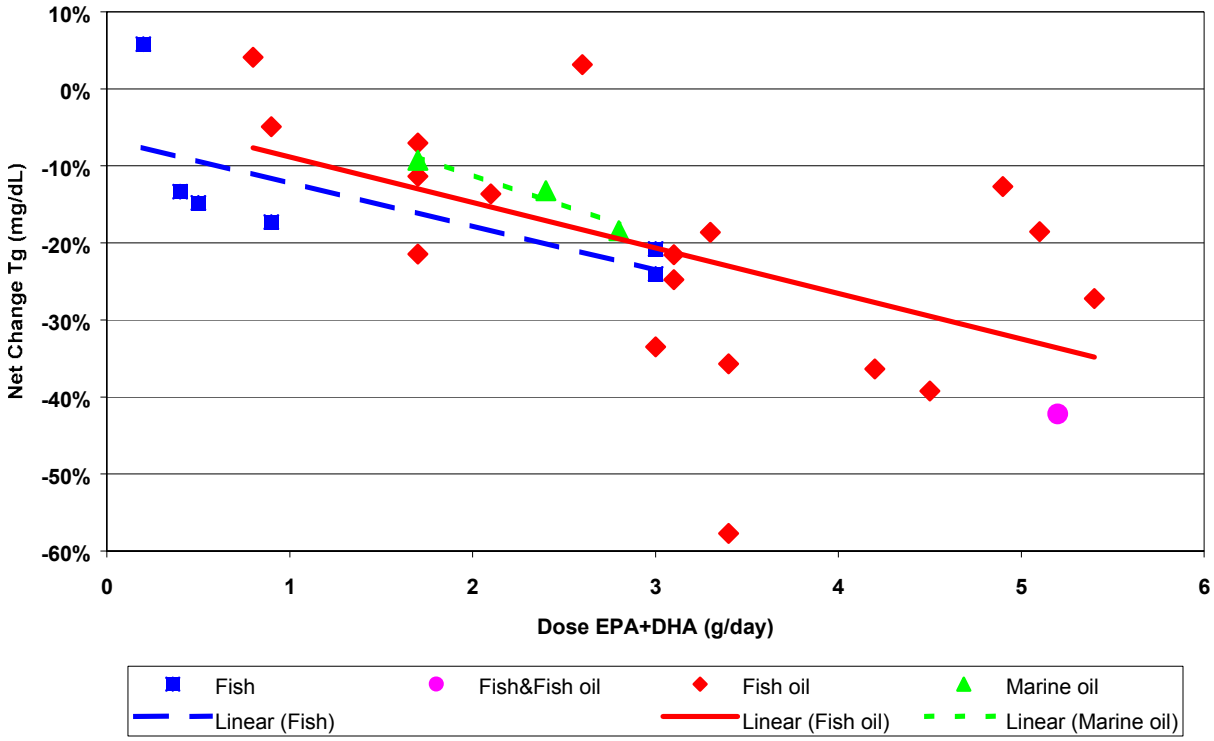


Dose and Source Effect

The 4 studies that compared different doses of marine oil supplements found that the greatest net decrease in Tg level occurred in study arms receiving the highest dose of EPA+DHA, although none of the articles reported whether there was a significant trend within the study. Across studies there was a clear trend toward greater percent decrease in Tg with higher doses, regardless of source (Figure 3.2). At least a 10% reduction in Tg was found in most studies with doses of at least 1.7 g per day of marine oil supplementation. Most study arms with doses of at least 3 g per day of marine oil supplements resulted in at least a 20% reduction in Tg. Among the studies of dietary fish, only the 2 arms with high omega-3 fatty acid fish diets in Mori, et al. achieved at least a 20% reduction of Tg [71].

Grimsgaard et al., overall, found no difference in effect between purified EPA and purified DHA, although the net decreases in Tg were consistently greater in the DHA group than in the EPA group across quartiles of baseline Tg [66]. Across studies, and within the Mori et al. study [71], the source of the EPA+DHA, whether as a supplement or from dietary fish, does not appear to make a difference. In contrast, the effect of ALA is uncertain. The single study that evaluated pure ALA supplementation, Finnegan et al., found increases in Tg levels in subjects on both 4.5 g and 9.5 g per day of ALA margarine (the latter dose is not included in the summary table) [53]. Both Singh et al. and de Lorgeril et al. provided ALA in the context of a Mediterranean diet, which also included higher dietary fish intake [49,76].

Figure 3.2 Meta-regression of dose of EPA+DHA intake versus net change in triglycerides (Tg). Each point represents an individual study or study arm. Separate simple regressions were performed for each oil source type (except for the individual study arm of combined fish and fish oil). Marine oils include non-fish animal sources including Minke whale and seal. Regression not adjusted for baseline Tg or study size.



Exposure Duration

The effect of duration of intervention or exposure was somewhat inconsistent among the 4 studies that reported data on Tg levels at different time points in studies of omega-3 fatty acids. Hanninen et al. found progressive decreases of Tg at 5 and 12 weeks in group of subjects consuming higher amounts of fish [67]. Similarly, Nilsen et al found progressive decreases in men, but not in a small group of women, at 6 weeks, 6 months and 12 months [73]. Sirtori et al. found that the effect of lower dose fish oil supplementation to reduce Tg occurred by 2 months and remained stable at 4 and 6 months [77]. In contrast, Finnegan et al. reported a significant decrease (15%) in mean Tg levels after 2 months which was not sustained at 6 months in the EPA+DHA arms [53]. Across studies, there is no apparent correlation between study duration and fish oil supplement effect, even after grouping studies by fish oil dosage.

Sustainment of Effect

No study reported data on an effect after ceasing omega-3 fatty acid treatment.

Lipoprotein(a)

(Table 3.6)

Lipoprotein(a) [Lp(a)] consists of an LDL core covalently bound to a plasminogen-like glycoprotein, apolipoprotein(a) [82]. Elevated levels of Lp(a) are an independent risk factor for atherosclerotic disease, possibly by promoting thrombosis. Lp(a) levels are largely determined by genetic polymorphism, specifically the number of K-IV repeats. Steroid hormones, and thus menopause, affect levels. There is a very large range of Lp(a) levels, from less than 0.1 mg/dL to more than 300 mg/dL and the distribution can be highly skewed. Treatments available to lower Lp(a) levels include niacin and hormone replacement therapy (in post-menopausal women).

We found 23 studies that met eligibility criteria and reported data on the effect of omega-3 fatty acids on Lp(a) levels (See Table 3.1). Of these, we analyzed the 14 randomized trials. All but 2 were parallel trials. The source of fatty acids was marine oil supplements in 12 studies, dietary fish in 1 study and Mediterranean diet in 1 study.

Overall Effect [49,55,58,62,83-92]

Across the 14 studies there is no consistent effect on Lp(a) levels of omega-3 fatty acid consumption compared to control. In approximately one-third of the studies the omega-3 fatty acid study arms had a net increase in Lp(a) level compared to control; in the remaining studies the net decrease in Lp(a) level was generally small and non-significant. Only 2 studies reported a statistically significant difference between the effect of omega-3 fatty acid and control, both of which found a net decrease in Lp(a). However, the variability of Lp(a) levels among subjects within all the studies resulted in wide confidence intervals which limited the likelihood of statistically significant findings.

Sub-populations

The 5 studies that evaluated generally healthy subjects found no consistent effect of omega-3 fatty acids on Lp(a). Marckmann et al. found a large net increase of Lp(a) with fish oil supplement use and Deslypere et al. found a large net increase of Lp(a) in 1 of 3 treatment arms [85,89]. The remaining studies (and study arms) reported generally small effects, which were not uniform in direction. Five studies evaluated subjects with known CVD, one of which included only patients with hypertriglyceridemia on simvastatin. The apparent large decrease in Lp(a) in the latter study, Durrington et al., occurred because the median Lp(a) level rose by less in the fish oil supplement group than the corn oil group [86]. Again no consistent effect was seen. In the only study of diabetic subjects, Luo et al. found a statistically significant net reduction of Lp(a) of about 20% with fish oil supplementation [88]. The 4 studies of subjects with dyslipidemia (including the one with subjects with CVD on simvastatin) all found that subjects on marine oil supplements had a net decrease in Lp(a) compared to control; however, none of the changes was significant.

Table 3.6 Effects of omega-3 fatty acids on lipoprotein (a) (mg/dL) in randomized trials (4 weeks to 14 months)

Author, Year	Omega-3 Fatty Acid Arm [a]				Control		Results [b]			Quality [c]				
	N	Source		g/d	N	Source	Base	Net Δ	*P*	Summary	Jadad	Allocation Conceal	Applicability [d]	
		DHA/EPA Oils												
Deslypere, 1992	14	Fish oil	T	3.4	14	Olive oil	22.5	+10.3	NS	B	2	Un	GEN	I
	15	Fish oil	T	2.2			27.2	+2.3	NS					
	15	Fish oil	T	1.1			22.1	+4.9	NS					
Alaswad, 1999	11	Fish oil	ED	3.4	12	Calcium gluconate	7.8	-1.1	NS	B	2	Un	DysLip	II
Prisco, 1994	10	Fish oil	ED	3.4	10	Olive oil	10.2	-0.9	NS	B	3	Un	GEN	II
Eritsland, 1995a	214 [e]	Fish oil	ED	3.3	219 [e]	No oil	[5.5]	[0]	NS	B	2	Ad	CVD	II
	66 [f]				50 [f]		[29.7]	[-1.5]	.02					
Brox, 2001 [g]	38	Cod liver oil	ED	3.3	37	No oil	18.5	-1.7	NS	C	1	Un	DysLip	I
	37	Seal oil	ED	2.6			16.3	-1.9	NS					
Durrington, 2001	30	Fish oil	ED	3.2	29	Corn oil	[10.5]	[-6.8]	NS	A	4	Un	CVD DysLip	II
Conquer, 1999	9	Seal oil	ED	3.0	10	Evening primrose	1.6	+0.1	NS	A	4	Un	GEN	II
Swahn, 1998	26	Fish oil	ED	2.9	27	Corn oil	30.8	-0.7	NS	B	5	Un	CVD	II
Hamazaki, 1996	13	DHA-rich Fish oil	ED	1.7-2.0 [h]	11	Corn oil	120	0	NS	B	4	Un	GEN	II
Luo, 1998	10 [i]	Fish oil	ED	1.8	10 [i]	Sunflower	17	-3	<.02	B	3	Un	DM II	II
Marckmann, 1997	22	Fish oil margarine	T	0.9	24	Sunflower margarine	[3.6]	[+3.0]	NS	B	3	Un	GEN	II
Nenseter, 2000	34	Fish powder	ED	0.2	36	Cellulose	[13.5]	[-0.8]	NS	B	3	Un	DysLip	I
		Fish and Mediterranean Diets												
de Lorgeril, 1994	171 [j]	Mediterranean/ Canola margarine	A	0.8% Kcal	168 [k]	Regular	28	+6	NS	C	2	Un	CVD	II
Schaefer, 1996	11	High fish	ED	0.7% Kcal	11	Low fish	38	-3	NS	C	1	Un	GEN	I

a A = ALA; D = DHA; E = EPA; ED = EPA+DHA; T = Total omega-3 fatty acids.

b Base = baseline level in treatment arm. Numbers in brackets are median values.; Net Δ = net difference in change in omega-3 fatty acids arm compared with the change in control arm; for studies with numbers in brackets, the net difference was estimated by subtracting the final median value from the baseline median value; see Methods; *P* = *P* value of difference between treatment and control arms; NS = not statistically significant.

c A = good quality; B = fair quality; C = poor quality; Jadad = Jadad Score (0-5, based on randomization, double blinding, and dropouts); Ad = adequate allocation concealment; In = inadequate allocation concealment; Un = allocation concealment unclear. See Methods.

d CVD = history of cardiovascular disease; DM I/II = diabetes mellitus type 1 or 2; DysLip = dyslipidemia; GEN = general, healthy population; N/IDDM = (non-) insulin dependent diabetes mellitus. I = broadly applicable; II = applicable to sub-group; III = narrow applicability. See Methods.

e Baseline Lp(a) < 20 mg/dL.

f Baseline Lp(a) >= 20 mg/dL.

g Data missing from article provided by study author.

h Depending on body weight.

i Cross-over study.

j Baseline data based on 289 subjects.

k Baseline data based on 295 subjects.

Eritsland et al. found that the effect on Lp(a) was not related to age or sex [87]. The 2 studies that excluded pre-menopausal women both found small, non-significant, net reductions in mean Lp(a) with fish oil supplements or fish diet [58,83]. The 4 studies of men generally found small, non-significant, net increases in Lp(a) [84,85,89,91]. No study included only women.

Covariates

As shown in the summary table, Eritsland et al. found a differential effect of omega-3 fatty acids based on baseline Lp(a) level in patients referred for coronary artery bypass graft surgery [87]. Those with Lp(a) in the upper quintile (≥ 20 mg/dL) had a small but significant absolute and net reduction in Lp(a), while the remaining subjects did not. A similar comparison between subjects with elevated baseline Tg (≥ 245 mg/dL) and those with lower Tg found no difference in effect.

Dose and Source Effect

Only 2 studies directly compared different doses of fish oil supplements or different oils. Deslypere et al. reported no effect on Lp(a) at any of 3 doses of fish oil supplements, although the mean Lp(a) level rose by almost 50% after 1 year in subjects on the highest dose [85]. Brox et al. found no difference between similar doses of cod liver oil and seal oil supplements [62]. Across studies no differences could be discerned based on marine oil dose or omega-3 fatty acid-rich diet.

Exposure Duration

Two studies reported Lp(a) data at different time periods. de Lorgeril et al. found no difference in effect on Lp(a) at 8, 52, and 104 weeks in a study of Mediterranean diet [49]. Prisco et al. also found no difference in effect at 2 and 4 months in a study of fish oil supplements [91]. Across studies there is no apparent relationship between effect and duration of intervention or exposure.

Sustainment of Effect

Both Prisco et al. and Deslypere et al. reported no difference between Lp(a) levels while subjects were on fish oil supplements and at multiple time points up to 6 months after stopping supplementation [85,91].

Apolipoprotein A-I

(Table 3.7)

Apolipoprotein A-I (apo A-I) is the major apolipoprotein of HDL. It serves as a cofactor for enzymes that metabolize HDL in plasma. Apo A-I levels are strongly correlated with HDL cholesterol levels, but ratios of HDL to apo A-I do vary. While the effect of omega-3 fatty acids on lipoprotein-associated cholesterol and apolipoprotein assays are of interest, unlike cholesterol

levels, apolipoprotein assays, which are antibody specific and are not standardized, are not as amenable to cross-study comparisons. Furthermore, there are no data to suggest that apolipoprotein levels are more predictive of CVD risk than lipoprotein cholesterol levels.

We found 61 studies that met eligibility criteria and reported data on the effect of omega-3 fatty acids on apo A-I levels (See Table 3.1). Of these, we analyzed the 27 randomized trials with data on at least 20 subjects in parallel trials and 15 subjects in crossover trials who consumed omega-3 fatty acids.

Table 3.7 Effects of omega-3 fatty Acids on apolipoprotein A-I (mg/dL) in randomized trials (4 weeks to 2 years)

Author, Year	Omega-3 Fatty Acid Arm [a]			Control		Results [b]			Quality [c]				
	N	Source	g/d	N	Source	Base	Net Δ	*P*	Summary	Jadad	Allocation Conceal	Applicability [d]	
		DHA/EPA Oils											
Wilt, 1989	38 [e]	Fish oil	ED 6.0	38 [e]	Safflower	151	-4	NS	B	4	Un	DysLip	III
Green, 1990	27 [e]	Fish oil	ED 5.2	27 [e]	Corn/Olive	113	-10	NS	B	4	Un	DysLip	II
Bonaa, 1992	71	Fish oil	ED 5.1	74	Corn oil	155	-7	<.05	B	4	Un	DysLip	I
Balestrieri, 1996	14 [e]	Fish oil	ED 5.1	38 [e]	Olive oil	116	-2	NS	B	3	Un	DysLip	III
Jensen, 1989	18 [e]	Cod liver oil	ED 4.6	18 [e]	Olive oil	134	-6	NS	B	4	Un	IDDM	II
Sirtori, 1992	12 [e]	Fish oil	ED 4.5	12 [e]	No oil	132	-4	nd	C	2	Un	DysLip	II
Schectman, 1989 [f]	18 [e]	Fish oil	ED 4.0	18 [e]	Safflower	117	-5	NS	B	2	Un	DysLip	II
Schectman, 1988 [f]	13 [e]	Fish oil	ED 4.0	13 [e]	Safflower	114	-10	NS	B	2	Un	NIDDM	II
Grimsgaard, 1997	75	Purified EPA	E 3.8	77	Corn oil	138	-4	.02 [g]	A	5	Un	GEN	I
	72	Purified DHA	D 3.7			138	+2	NS [g]					
Harris, 1997	22	Fish oil	ED 3.4	20	Corn oil	132	+1	NS	B	3	Un	DysLip	II
Nordoy, 1998	21	Fish oil	ED 3.4	20	Corn oil	142	+1	NS	B	4	Un	DysLip	I
Deslypere, 1992	14	Fish oil	T 3.4	14	Olive oil	137	-17	NS	B	2	Un	GEN	III
	15	Fish oil	T 2.2			139	-7	NS					
	15	Fish oil	T 1.1			137	-9	NS					
Chan, 2002	12	Fish oil	ED 3.4	12	Corn oil	118	+5	NS [h]	B	3	Un	DysLip	II
	11	Fish oil & Atorvastatin		12	Corn oil & Atorvastatin	128	+3						
Eritsland, 1995b	178	Fish oil	ED 3.3	174	No oil	124	+2	NS	B	2	Ad	CVD	II
Brox, 2001 [i]	38	Cod liver oil	ED 3.3	37	No oil	160	0	NS	C	1	Un	DysLip	I
	37	Seal oil	ED 2.6			160	+10 [j]	NS					
Durrington, 2001	30	Fish oil	ED 3.2	29	Corn oil	90	-6	NS	A	4	Un	CVD DysLip	II
McGrath, 1996	23 [e]	Fish oil	ED 3.0	23 [e]	Olive oil	119	+2	NS	A	4	Un	DM II	II
Nikkila, 1991	32 [e]	Fish oil	ED 2.4	32 [e]	Corn oil	109	-2	NS	B	3	Un	CVD DysLip	II
Luo, 1998	10 [e]	Fish oil	ED 1.8	10 [e]	Sunflower	148	+1	NS	B	3	Un	DM II	II
Marckmann, 1997	23	Fish oil margarine	T 0.9	24	Sunflower margarine	149	-2	NS	B	3	Un	GEN	II

Continued

Table 3.7 Effects of omega-3 fatty Acids on apolipoprotein A-I (mg/dL) in randomized trials (continued)

Author, Year	Omega-3 Fatty Acid Arm [a]				Control		Results [b]			Quality [c]				
	N	Source		g/d	N	Source	Base	Net Δ	*P*	Summary	Jadad	Allocation Conceal	Applicability [d]	
		Fish and Mediterranean Diets												
Hanninen, 1989	19	Fish (3.8/week)	ED	0.9	18	0.4 Fish/week	113	0	NS	B	2	Un	GEN	III
	22	Fish (2.3/week)	ED	0.5			117	+2	NS					
	21	Fish (1.5/week)	ED	0.4			121	-9	NS					
	20	Fish (0.9/week)	ED	0.2			118	0	NS					
Agren, 1988	14	Fish (3.7/week)	ED	0.8	12	0.25 Fish/week	126	-14	<.01	B	3	Un	GEN	III
	15	Fish & low SFA					123	-3	NS					
Agren, 1991	20	Fish (5/week)	ED	0.75	15	Regular	149	+8	NS	B	2	Un	GEN	III
	20	Fish (5/week) [k]			23	Regular [k]	159	0	NS					
de Lorgeril, 1994	171 [L]	Mediterranean/ Canola margarine	A	0.8% Kcal	168 [m]	Regular	124	-12	NS	C	2	Un	CVD	II
		Combinations												
Cobiac, 1991	13	Fish oil	ED	4.6	6	Olive, Palm, Safflower oil	117 [n]	+1	NS	B	2	Un	GEN	II
	12	Fatty Fish diet	ED	4.5			120 [n]	0	NS					
Silva, 1996	20	Fish oil	ED	3.6	--	--	159	-28 [o]	nd [p]	B	3	Un	DysLip	II
	15	Soya oil	A	0.8 [q]			184	-33 [o]	nd [p]					
Agren, 1996	14	Fish oil	ED	2.3	14	No oil	125	-8	NS	B	3	Un	GEN	III
	14	Algae DHA oil	D	1.7			128	+1	NS					
	13	Fatty Fish diet	ED	1.1			120	+1	NS					

nd = no data

a A = ALA; D = DHA; E = EPA; ED = EPA+DHA; T = Total omega-3 fatty acids.

b Base = baseline level in treatment arm; Net Δ = net difference in change in omega-3 fatty acids arm compared with the change in control arm, see Methods; *P* = *P* value of difference between treatment and control arms; NS = not statistically significant.

c A = good quality; B = fair quality; C = poor quality; Jadad = Jadad Score (0-5, based on randomization, double blinding, and dropouts); Ad = adequate allocation concealment; In = inadequate allocation concealment; Un = allocation concealment unclear. See Methods.

d CVD = history of cardiovascular disease; DM I/II = diabetes mellitus type 1 or 2; DysLip = dyslipidemia; GEN = general, healthy population; N/IDDM = (non-) insulin dependent diabetes mellitus. I = broadly applicable; II = applicable to sub-group; III = narrow applicability. See Methods.

e Cross-over study.

f Unclear if 2 studies by Schectman et al. [93,94] are independent of each other. Possible overlap of up to 6 subjects with NIDDM and hypertriglyceridemia.

g *P*=.0008 for difference in effect of EPA and DHA.

h Main effect.

i Data missing from article provided by study author.

j Only 2 significant digits reported. 10 mg/dL is smallest unit of change possible.

k Recommended aerobic exercise for 30 minutes 3 times a week.

L Baseline data based on 289 subjects.

m Baseline data based on 295 subjects.

n Units were not reported

o Pre-post difference (not compared to control).

p NS between treatments.

q No data on ALA amount. We assumed 7 g ALA per 100 g oil. 12 g oil.

Overall Effect [48,49,52,62,66,67,85,86,88,89,93-109]

Across the 27 studies, effects of omega-3 fatty acids on apo A-I levels were generally heterogeneous but small. Most studies found a small net change in apo A-I with omega-3 fatty acid consumption. Three-quarters of studies found net changes between –5% and +5% (–7 to

+10 mg/dL). No study found a large net increase in apo A-I level. A small number of studies found larger net decreases of up to 18% reductions (–33 mg/dL).

Sub-populations

Eight studies evaluated healthy people, all single-sex groups (7 male[66,85,89,95,97,100,110], 1 female[96]), mostly of university students. Four studies evaluated diabetic patients. Thirteen studies evaluated patients with dyslipidemia, 2 of which were also of patients with CVD. There was one additional study of patients with CVD. There were no clear patterns of treatment effect or differences in effect among the sub-populations.

Covariates

Silva et al. reported that sex, body mass index, hypertension, and non-insulin dependent diabetes did not affect the fish oil or soya oil supplement effect on lipid parameters including apo A-I in hyperlipidemic subjects [107]. No other study evaluated correlations or sub-analyses based on apo A-I. Agren et al. (1988) compared the effect of daily fish with daily fish with a low saturated fat diet in male university students [95]. Among subjects on a fish and low saturated fat diet, apo A-I levels remained essentially unchanged compared to those on a regular diet. In contrast, subjects on a fish diet who were not told to lower their saturated fat intake had a significant net decrease in apo A-I that was among the largest net decreases across studies. However, no comparison was made between the 2 treatment groups, nor were any explanations for the difference examined or discussed. Three studies compared fish oil to placebo oil supplements in dyslipidemic patients who were all taking either atorvastatin or simvastatin [98,99,106]. The effects of fish oil supplementation on apo A-I were small in all 3 studies. The effects were not uniform in direction.

Dose and Source Effect

Neither Deslypere et al. nor Hanninen et al. reported a dose dependent effect on apo A-I of either fish oil supplements or different frequencies of fish meals [67,85]. No dose effect was seen across studies of EPA+DHA either.

Five studies compared different sources of omega-3 fatty acids. Grimsgaard et al. found a small but significant net decrease in apo A-I with purified EPA compared to a smaller, non-significant, net increase with purified DHA; the difference between the 2 omega-3 fatty acids was statistically significant ($P = .008$) [66]. Brox et al. compared 2 sources of marine oil supplements: cod liver and seal oil [62]. No effect was found with either treatment. Cobiac et al. found no treatment effect with either fish oil supplementation or with a fatty fish diet [100]. Silva et al. found similarly large, significant reductions in apo A-I level in subjects taking either fish oil or soya oil supplements; however, no non-omega-3 fatty acid was used as a control [107]. Agren et al. (1996) compared fish oil supplementation, algae DHA oil supplementation, and fatty fish diet and also found no difference in effect on apo A-I among the groups [97].

Exposure Duration

Two studies reported apo A-I levels at multiple time points. Neither Hanninen et al. nor de Lorgeril et al. found any time-related effects of omega-3 fatty acids on apo A-I, at 5 and 12 weeks, and 8, 52, and 104 weeks, respectively [49,67].

Sustainment of Effect

Three studies followed subjects after stopping the intervention. Jensen et al. and Deslypere et al. found no change in apo A-I levels 8 weeks and 6 months, respectively, after stopping fish oil supplements [85,103]. In contrast, Agren et al. (1988) reported that 5 months after a 15 week trial of dietary fish apo A-I levels remained at lowered levels in the fish diet group who had no limitation of saturated fat; however, they do not indicate what these students' diets were at subsequent follow-up [95].

Apolipoprotein B, Apolipoprotein B-100, and LDL Apolipoprotein B

(Tables 3.8 and 3.9)

Apolipoprotein (apo) B has 2 major subtypes, B-100 and B-48. Apo B-100 is associated with lipoprotein particles of hepatic origin, specifically very low, intermediate, and low density lipoproteins (VLDL, IDL, LDL). Its major function is to serve as a ligand for the receptor that clears these particles from the bloodstream. During the conversion of VLDL to LDL in the circulation, only apo B-100 remains on LDL. Measures of LDL apo B represent the portion of total blood apoB-100 that is associated with the LDL subfraction. There is 1 apo B-100 molecule per LDL particle. A discordance in LDL apoB-100 and LDL cholesterol levels implies a change in the composition of the LDL particle. Total apo B is thus indicative of VLDL, IDL and LDL levels, while apo B-100 and LDL apo B are indicative specifically of LDL levels. While the effect of omega-3 fatty acids on lipoprotein-associated cholesterol and apolipoprotein assays are of interest, unlike cholesterol levels, apolipoprotein assays, which are antibody specific and are not standardized, are not as amenable to cross-study comparisons. Furthermore, there are no data to suggest that apolipoprotein levels are more predictive of CVD risk than lipoprotein cholesterol levels.

We found 52 studies that met eligibility criteria and reported data on the effect of omega-3 fatty acids on total apo B levels, and 11 studies that reported data on either apo B-100 or LDL apo B (See Table 3.1). Of these, we analyzed the 25 randomized trials of apo B that had data on at least 20 subjects in parallel trials and 10 subjects in crossover trials who consumed omega-3 fatty acids. We also analyzed the 10 studies of apo B-100 or LDL apo B, all of which were randomized.

Overall Effect

Total apo B (Table 3.8) [48,49,53,66,67,71,85,86,88-90,93,95-101,103-106,108,109]. Across the 25 studies, we found little consistency in the effect of omega-3 fatty acids on apo B levels. About half the

studies found a small net increase and half a small net decrease in apo B levels. Only 2 studies found significant changes in individual study arms, but Deslypere et al. found a significant decrease and Mori et al. found a significant increase [71,85].

Table 3.8 Effects of omega-3 fatty acids on apolipoprotein B (mg/dL) in randomized trials (4 weeks to 2 years)

Author, Year	Omega-3 Fatty Acid Arm [a]				Control		Results [b]			Quality [c]				
	N	Source		g/d	N	Source	Base	Net Δ	*P*	Summary	Jadad	Allocation Conceal	Applicability [d]	
		DHA/EPA Oils												
Wilt, 1989	38 [e]	Fish oil	ED	6.0	38 [e]	Safflower	112	+5	NS	B	4	Un	DysLip	III
Green, 1990	27 [e]	Fish oil	ED	5.2	27 [e]	Corn/Olive	122	-5	NS	B	4	Un	DysLip	II
Bonaa, 1992	71	Fish oil	ED	5.1	74	Corn oil	153	-1	NS	B	4	Un	DysLip	I
Balestrieri, 1996	14 [e]	Fish oil	ED	5.1	14 [e]	Olive oil	205	+1	NS	B	3	Un	DysLip	III
Jensen, 1989	18 [e]	Cod liver oil	ED	4.6	18 [e]	Olive oil	109	+6	NS	B	4	Un	IDDM	II
Sirtori, 1992	12 [e]	Fish oil	ED	4.5	12 [e]	No oil	167	0	NS	C	2	Un	DysLip	II
Schectman, 1988	13 [e]	Fish oil	ED	4.0	13 [e]	Safflower	99	+7	NS	B	2	Un	NIDDM	II
Grimsgaard, 1997	75	Purified EPA	E	3.8	77	Corn oil	101	-5	NS	A	5	Un	GEN	I
	72	Purified DHA	D	3.7			100	-3	NS					
Nordoy, 1998	21	Fish oil	ED	3.4	20	Corn oil	108	+1	NS	B	4	Un	DysLip	I
Deslypere, 1992	14	Fish oil	T	3.4	14	Olive oil	89	-8	<.05	B	2	Un	GEN	III
	15	Fish oil	T	2.2			85	-1	NS					
	15	Fish oil	T	1.1			91	-2	NS					
Chan, 2002	12	Fish oil	ED	3.4	12	Corn oil	128	-4	NS [f]	B	3	Un	DysLip	II
	11	Fish oil & Atorvastatin			12	Corn oil & Atorvastatin	134	-8						
Durrington, 2001	30	Fish oil	ED	3.2	29	Corn oil	96	+5	NS	A	4	Un	CVD DysLip	II
McGrath, 1996	23 [e]	Fish oil	ED	3.0	23 [e]	Olive oil	95	+1	NS	A	4	Un	DM II	II
Nikkila, 1991	32 [e]	Fish oil	ED	2.4	32 [e]	Corn oil	122	+3	NS	B	3	Un	CVD DysLip	II
Luo, 1998	10 [e]	Fish oil	ED	1.8	10 [e]	Sunflower	138	+10	NS	B	3	Un	DM II	II
Marckmann, 1997	23	Fish oil margarine	T	0.9	24	Sunflower margarine	113	+1	NS	B	3	Un	GEN	II
Nenseter, 2000	34	Fish powder	ED	0.2	36	Cellulose	133	+2	NS	B	3	Un	GEN	II
		Fish and Mediterranean Diets												
Hanninen, 1989	19	Fish (3.8/week)	ED	0.9	18	0.4 Fish/week	93	-9	nd	B	2	Un	GEN	III
	22	Fish (2.3/week)	ED	0.5			78	-2	nd					
	21	Fish (1.5/week)	ED	0.4			80	-5	nd					
	20	Fish (0.9/week)	ED	0.2			78	0	nd					
Agren, 1988	14	Fish (3.7/week)	ED	0.8	12	0.25 Fish/week	70	-2	NS	B	3	Un	GEN	III
	15	Fish & low SFA					63	-3	NS					
Agren, 1991	20	Fish (5/week)	ED	0.75	15	Regular	64	+2	NS	B	2	Un	GEN	III
	20	Fish (5/week) [g]			23	Regular [g]	67	+5	NS					
de Lorgeril, 1994	171 [h]	Mediterranean/ Canola margarine	A	0.8% Kcal	168 [i]	Regular	152	-1	NS	C	2	Un	CVD	II

Table 3.8 Effects of omega-3 fatty acids on apolipoprotein B (mg/dL) in randomized trials (continued)

Author, Year	Omega-3 Fatty Acid Arm [a]				Control		Results [b]				Quality [c]				
	N	Source		g/d	N	Source	Base	Net Δ		P	Summary	Jadad	Allocation Conceal	Applicability [d]	
		Combinations													
Mori, 1994	17	Fish oil & Fish diet [j]	ED	5.2	18	Olive/Palm/ Safflower 40% fat diet	143 [k]	+5 [L]	NS		B	2	Un	GEN	II
	16	Fish oil	ED	4.2				+9 [L]	<.05						
	17	Fish oil	ED	2.1				+12 [L]	<.05						
	17	Fish diet [j] & Placebo oil	ED	3.0				+6 [L]	NS						
	18	Fish diet [j] & Placebo oil	ED	3.0	17	Oil 30% fat diet		+1 [L]	NS						
Cobiac, 1991	13	Fish oil	ED	4.6	6	Olive, Palm, Safflower oil	99 [m]	+6	NS		B	2	Un	GEN	II
	12	Fatty Fish diet	ED	4.5			100 [m]	-1	NS						
Agren, 1996	14	Fish oil	ED	2.3	14	No oil	72	-3	NS		B	3	Un	GEN	III
	14	Algae DHA oil	D	1.7			71	-3	NS						
	13	Fatty Fish diet	ED	1.1			75	0	NS						
Finnegan, 2003	31	Fish oil margarine/ Fish oil	ED	1.7	30	Sunflower margarine	176 [n]	+1	NS		A	4	Un	DysLip	I
	30	Fish oil margarine	ED	0.8			174 [o]	+3	NS						
	30	Rapeseed/Linseed margarine	A	4.5			178 [p]	+1	NS						

nd = no data

a A = ALA; D = DHA; E = EPA; ED = EPA+DHA; T = Total omega-3 fatty acids.

b Base = baseline level in treatment arm; Net Δ = net difference in change in omega-3 fatty acids arm compared with the change in control arm, see Methods; *P* = *P* value of difference between treatment and control arms; NS = not statistically significant.

c A = good quality; B = fair quality; C = poor quality; Jadad = Jadad Score (0-5, based on randomization, double blinding, and dropouts); Ad = adequate allocation concealment; In = inadequate allocation concealment; Un = allocation concealment unclear. See Methods.

d CVD = history of cardiovascular disease; DM I/II = diabetes mellitus type 1 or 2; DysLip = dyslipidemia; GEN = general, healthy population; N/IDDM = (non-) insulin dependent diabetes mellitus. I = broadly applicable; II = applicable to sub-group; III = narrow applicability. See Methods.

e Cross-over study.

f Main effect.

g Recommended aerobic exercise for 30 minutes 3 times a week.

h Baseline data based on 289 subjects.

i Baseline data based on 295 subjects.

j Omega-3 fatty acid from fish diet is approximate, assuming that each of 4 different fish was consumed equally.

k Mean baseline value for all subjects combined.

L Estimated from graph.

m Units were not reported.

n Reported as 1.76 mmol/L.

o Reported as 1.74 mmol/L.

p Reported as 1.78 mmol/L.

Apo B-100 (Table 3.9, top) [50,52,62,107] and LDL apo B (Table 3.9, bottom) [93,94,108,111-113]. The 4 studies of apo B-100 found a range of effects with omega-3 fatty acid consumption. Two found a decreases in level of less than 5%; the other 2 studies found net increases of 2% and 15%. In contrast, large, significant net increases in LDL apo B were found in 4 of 6 studies (20 to 45 mg/dL).

Table 3.9 Effects of omega-3 fatty acids on apolipoprotein B-100 and LDL apolipoprotein B (mg/dL) in randomized trials (1 month to 14 months)

Author, Year	Omega-3 Fatty Acid Arm [a]				Control		Results [b]			Quality [c]				
	N	Source		g/d	N	Source	Base	Net Δ	*P*	Summary	Jadad	Allocation Conceal	Applicability [d]	
Apo B-100														
		DHA/EPA Oils												
Eritsland, 1995b	178	Fish oil	ED	3.3	174	No oil	182	+3	NS	B	2	Ad	CVD	II
Brox, 2001 [e]	38	Cod liver oil	ED	3.3	37	No oil	200	-10 [f]	NS	C	1	Un	DysLip	I
	37	Seal oil	ED	2.6			200	-10 [f]	NS					
DeLany, 1990 [g]	5	Fish oil	ED	2	5	No oil	62	+9	NS	C	1	Un	GEN	III
		Combinations												
Silva, 1996	20	Fish oil	ED	3.6	--	--	188	-3 [h]	nd [i]	B	3	Un	DysLip	II
	15	Soya oil	A	0.8 [j]			222	-5 [h]	nd [i]					
LDL Apo B														
		DHA/EPA Oils												
Deck, 1989	8 [k]	Fish oil	ED	4.6	8 [k]	Corn oil	96	+25	<.05	B	5	Un	DysLip	II
Sirtori, 1992	12 [k]	Fish oil	ED	4.5	12 [k]	No oil	157	+2	NS	C	2	Un	DysLip	II
Schectman, 1989 [L]	15 [k]	Fish oil	ED	4.0	15 [k]	Safflower	92	+20	nd [m]	C	2	Un	DysLip	II
Schectman, 1988 [L]	13 [k]	Fish oil	ED	4.0	13 [k]	Safflower	82	+9	<.05	B	2	Un	NIDDM	II
Radack, 1990	10	Fish oil	ED	2.2	8	Olive oil	100	+45	<.05	B	5	Un	DysLip	II
	7	Fish oil	ED	1.1			95	+29	<.05					
Radack, 1991	33 [k]	Fish oil	ED	2.0	33 [k]	Safflower oil	249	-6	NS	B	5	Ad	CVD	II

nd = no data

a A = ALA; D = DHA; E = EPA; ED = EPA+DHA; T = Total omega-3 fatty acids.

b Base = baseline level in treatment arm; Net Δ = net difference in change in omega-3 fatty acids arm compared with the change in control arm, see Methods; *P* = *P* value of difference between treatment and control arms; NS = not statistically significant.

c A = good quality; B = fair quality; C = poor quality; Jadad = Jadad Score (0-5, based on randomization, double blinding, and dropouts); Ad = adequate allocation concealment; In = inadequate allocation concealment; Un = allocation concealment unclear. See Methods.

d CVD = history of cardiovascular disease; DM I/II = diabetes mellitus type 1 or 2; DysLip = dyslipidemia; GEN = general, healthy population; N/IDDM = (non-) insulin dependent diabetes mellitus. I = broadly applicable; II = applicable to sub-group; III = narrow applicability. See Methods.

e Data missing from article provided by study author.

f Only 2 significant digits reported. 10 mg/dL is smallest unit of change possible.

g Possibly not randomized ("[S]ubjects were divided into… treatment groups so that initial mean cholesterol concentration of each group was similar.").

h Pre-post difference (not compared to control).

i NS between treatments.

j No data on ALA amount. Assume 7 g ALA per 100 g oil. 12 g oil.

k Cross-over study.

L Unclear if 2 studies by Schectman et al. [93,94] are independent of each other. Possible overlap of up to 6 subjects with NIDDM and hypertriglyceridemia.

m Increase in LDL apo B within fish oil arm was significant compared to baseline (*P*<.05).

Sub-populations

Total apo B. The heterogeneity of effects seen across all studies is apparent among the 10 studies of healthy populations (8 of which were in men[66,67,71,85,89,95,97,100] and one of which was in women[96]), the 10 studies of dyslipidemic populations (subjects in 2 of which also had CVD), and

the 3 studies of CVD populations (including those studies with subjects with dyslipidemia). The 4 studies of diabetics, one of which included insulin-dependent diabetics, all found small, non-significant, net increases in total apo B.

Apo B-100 and LDL apo B. The 2 apo B-100 studies of dyslipidemic patients reported small net decreases in apo B-100, while the study of patients undergoing coronary bypass surgery showed a small net increase and the study of healthy, male college students found a larger net increase in apo B-100. The 5 LDL apo B studies of dyslipidemic or diabetic subjects found generally large increases in LDL apo B, while the single study of hypertensive subjects showed a small net decrease.

Covariates

Total apo B. Nenseter et al. performed a subanalysis based on age of the effect of a low-omega-3 fatty acid fish powder [90]. Subjects between ages 30 and 52 years had a significantly greater rise in apo B level compared to subjects 53 to 70 years old; furthermore age negatively correlated with the rise in apo B ($r = -0.40$, $P < .04$). The authors also imply that the effect was not correlated with sex. Mori et al. performed a regression adjusting for change in weight and found a highly significant "group effect" increase in apo B with omega-3 fatty acids ($P < .01$) [71]. Agren et al. (1988), in a study of male university students, found no difference in effect between 2 fish diets that differed in the amount of low saturated fats [95]. Three studies compared fish oil to placebo oil supplements in dyslipidemic patients who were all taking either atorvastatin or simvastatin [98,99,106]. The effects of fish oil supplements on apo B were small in all. They were not uniform in direction.

Apo B-100 and LDL apo B. Silva et al. reported that any effect of fish oil and soya oil supplements on apo B was not correlated with sex, BMI, hypertension, or diabetes in hyperlipidemic patients [107]. Schectman et al. found that changes in LDL apo B did not correlate with baseline differences in diet or with individual changes in diet or body weight [93]. Other studies did not correlate findings with possible covariates. The small number of studies limits hypothesis generating of possible effect mediators across studies.

Dose and Source Effect

Total apo B. Among studies of fish oil supplements, Deslypere et al. found a significant net decrease in apo B in subjects on the highest dose of omega-3 fatty acids but smaller non-significant net decreases with smaller doses [85]. Among the individual study arms, apo B levels fell in the arm with a higher dose of fish oil but rose in the lower dose arms (and the olive oil arm). No dose effect was seen across fish oil supplement studies. Among studies of dietary fish, Hanninen et al. reported a trend in effect related to different frequencies of fish meals [67]. Subjects most frequently consuming fish had the largest, significant reduction in apo B (compared to baseline). Subjects with intermediate frequencies of fish consumptions (average of 1.5 and 2.3 meals per week) had smaller reductions in apo B with *P* values (compared to baseline) of less than .10. Subjects on only about 1 fish meal per week had a non-significant increase in apo B.

Five studies compared different sources of omega-3 fatty acids. Grimsgaard et al. found no difference in effect between purified EPA and purified DHA [66]. Mori et al. compared a variety of doses of fish oil supplements and combinations of dietary fish and supplemental fish oil, along with higher and lower percentage fat diets [71]. Overall, significant net increases in apo B were

seen in the subjects who consumed fish oil supplements and were on non-fish diets, but smaller, non-significant increases were seen in the subjects who were on fish diets, regardless of fish oil supplementation or percent fat in the diet. Cobiac et al. similarly found that subjects on fish oil supplement had a net increase in apo B while those on dietary fish had almost no change [100]. While neither change was statistically significant, there was a trend toward a difference between the 2 treatments ($P = .10$). In contrast, Agren et al. (1996) reported small non-significant net reductions in apo B with fish oil and algae DHA oil supplementation and no effect with fatty fish diet; although they do not comment on potential differences between groups [97]. Finally, Finnegan et al. reported no effects on apo B and no differences among people consuming different omega-3 fatty acids from margarine and/or supplements [53].

Apo B-100 and LDL apo B. Neither Brox et al. nor Silva et al. found a difference in effect of different omega-3 fatty acids on apo B-100 levels [62,107]. Radack et al. (1990) found a similar large increase in LDL apo B in 2 groups of hypertriglyceridemic patients consuming different doses of fish oil supplements [113]. While the increase was greater in the group consuming a higher dose of fish oil, no analysis was done to compare the effect in the 2 arms.

Exposure Duration

Total apo B. While the authors do not describe an effect of duration of fish consumption, the data at 5 and 12 weeks in Hanninen et al. may suggest that any effects of dietary fish on apo B do not occur until after 5 weeks [67]. At 5 weeks there were essentially no changes in apo B in any of the study arms, compared to significant and near significant reductions in arms with more frequent fish consumption. In de Lorgeril et al. a Mediterranean and ALA margarine diet had no effect on apo B at 8 weeks, 1 year, and 2 years.

Apo B-100 and LDL apo B. In their study of apo B-100, DeLany et al. found that while there was no difference in effect between 5 g fish oil supplementation and no oil at 5 weeks, there was a significant increase over time at 0, 2, and 5 weeks in subjects on fish oil supplements [50]. However, this analysis included 5 subjects who took 20 g fish oil supplements. There was also a small increase in apo B-100 levels in subjects not consuming oil supplements. Radack et al. (1990) reported no change in LDL apo B level between measurements at 8, 12, and 20 weeks [113].

Sustainment of Effect

Total apo B. Three studies followed subjects after stopping the intervention. Both Jensen et al. and Agren et al. (1988) found no change in apo B levels 8 weeks and 5 months, respectively, after stopping fish oil supplements [95,103]. Deslypere et al. found that 6 months after stopping supplements apo B levels rose to similar levels in all groups except those who had been on the lowest dose fish oil, although no analysis was performed on follow-up data [85].

Apo B-100 and LDL apo B. Although Radack et al. (1990) measured LDL apo B levels 4 weeks after stopping treatment [113], no study reported whether changes in apo B-100 or LDL apo B levels were sustained.

Blood Pressure

(Tables 3.10 and 3.11)

Hypertension is a well-known risk factor for atherosclerosis and cardiovascular disease. Recently the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 7) noted that the relationship between blood pressure and risk of cardiovascular events is continuous, consistent and independent of other factors.[25] The benefits to lowering blood pressure are evident even in people with "pre-hypertension" (blood pressure of 120-139/80-89).

We found 103 studies that met eligibility criteria and reported data on the effect of omega-3 fatty acids on blood pressure (See Table 3.1). In addition, we found a recent systematic review with a meta-regression of the blood pressure response to fish oil supplementation [114]. This thorough review touched on most of the major questions addressed by the current report, therefore this section relies primarily on the findings of Geleijnse et al. However, they explicitly excluded studies of diabetic patients. Therefore, we analyzed the 6 randomized trials with data on blood pressure in diabetic patients that had a minimum of 15 patients in parallel trials and 10 patients in crossover trials who consumed omega-3 fatty acids.

Meta-Regression [114]

Geleijnse et al. collected trials of fish oil supplementation and blood pressure through March 2001. Eligibility criteria were: (1) randomized design, (2) adult study population, and (3) publication after 1966. Trials were excluded if they included sick or hospitalized patients, including kidney disease and diabetic patients, or if the intervention was shorter than 2 weeks duration. A total of 36 trials with 50 omega-3 fatty acid study arms were analyzed. Of note, 6 of these studies did not meet our eligibility due to high omega-3 fatty acid dose (3), short washout period in crossover trial (2), or short study duration (1).

The range of trial duration was 3 to 52 weeks and doses of omega-3 fatty acids were less than 1.0 g/day in 1 trial, 1.0 to 1.9 g/day in 5 trials, 2.0 to 2.9 g/day in 4 trials, and 3.0 to 15.0 g/day in 26 trials.

The mean net reduction (controlling for placebo arms) in systolic and diastolic blood pressure, weighted for study size, was –2.1 mm Hg (95% confidence interval –3.2, –1.0) and –1.6 mm Hg (–2.2, –1.0), respectively. The mean reductions in systolic and diastolic blood pressures were somewhat smaller in the 22 double blinded studies. Data on univariate and multivariate weighted meta-regression analyses performed on study subgroups based on mean age, sex, mean baseline blood pressure, and mean body mass index are reported. Briefly, systolic and diastolic blood pressure reductions were significantly larger in older (mean age ≥ 45 years) than younger populations, and in hypertensive (blood pressure ≥ 140/90 mm Hg) compared to normotensive populations. A lack of studies in women precluded adequate analysis based on sex. Body mass index was not associated with blood pressure response to fish oil supplementation. In addition, trial duration and fish oil dose were not associated with effect.

Overall Effect in Diabetes Studies [115-120]

Across the 6 studies of diabetic patients, there were generally small, non-significant effects of fish oil supplements on systolic (Table 3.10) and diastolic (Table 3.11) blood pressure. Overall, these study results were similar to the findings of the meta-regression among non-diabetic populations in their small, but generally inconsistent net effects. One study reported a small significant reduction in mean diastolic pressure (–2 mm Hg) and 2 reported significant reductions in mean systolic pressure (–3 and –6 mm Hg).

Covariates

Haines et al., who found non-significant small net increases in blood pressure, reported that neither sex nor Hgb A_{1c} levels were related to the effect of fish oil supplements on blood pressure [115]. No study analyzed data based on age. Across studies there was no clear difference

Table 3.10 Effects of omega-3 fatty acids on systolic blood pressure (mm Hg) in randomized trials of diabetic subjects (6 weeks to 1 year)

Author, Year	Omega-3 Fatty Acid Arm [a]				Control		Results [b]			Quality [c]			
	N	Source		g/d	N	Source	Base	Net Δ	*P*	Summary	Jadad	Allocation Conceal	Applicability [d]
		DHA/EPA Oils											
Haines, 1986	19	Fish oil	ED	4.6	22	Olive oil	135	+1	NS	B	2	Ad	IDDM II
Rossing, 1996 [e]	14	Cod liver oil	ED	4.6	15	Olive oil	141	-3	NS	A	3	Un	IDDM II
Woodman, 2002 [e]	17	Purified EPA	E	3.8	16	Olive oil	137	0	NS	B	3	Un	DM II II
	17	Purified DHA	D	3.7			139	+7	NS				
Lungershausen, 1997	16	Fish oil	ED	3.4	16	Corn oil	139	-6	.04	B	4	Un	DM I&II II
Hendra, 1990	37	Fish oil	ED	3.0	37	Olive oil	145	+1	NS	B	4	Un	DM II I
Jain, 2002	25	Fish oil	ED	0.6	15	"Placebo"	127	-3	.0003	C	2	Un	DM II II

a A = ALA; D = DHA; E = EPA; ED = EPA+DHA; T = Total omega-3 fatty acids.

b Base = baseline level in treatment arm; Net Δ = net difference in change in omega-3 fatty acids arm compared with the change in control arm, see Methods; *P* = *P* value of difference between treatment and control arms; NS = not statistically significant.

c A = good quality; B = fair quality; C = poor quality; Jadad = Jadad Score (0-5, based on randomization, double blinding, and dropouts); Ad = adequate allocation concealment; In = inadequate allocation concealment; Un = allocation concealment unclear. See Methods.

d CVD = history of cardiovascular disease; DM I/II = diabetes mellitus type 1 or 2; DysLip = dyslipidemia; GEN = general, healthy population; N/IDDM = (non-) insulin dependent diabetes mellitus. I = broadly applicable; II = applicable to sub-group; III = narrow applicability. See Methods.

e Mean 24 hour ambulatory blood pressure.

Table 3.11 Effects of omega-3 fatty acids on diastolic blood pressure (mm Hg) in randomized trials of diabetic subjects (6 weeks to 1 year)

Author, Year	Omega-3 Fatty Acid Arm [a]				Control		Results [b]			Quality [c]			
	N	Source		g/d	N	Source	Base	Net Δ	*P*	Summary	Jadad	Allocation Conceal	Applicability [d]
		DHA/EPA Oils											
Haines, 1986	19	Fish oil	ED	4.6	22	Olive oil	81	+2	NS	B	2	Ad	IDDM II

Rossing, 1996 [e]	14	Cod liver oil	ED	4.6	15	Olive oil	82	-1	NS	A	3	Un	IDDM	II
Woodman, 2002 [e]	17	Purified EPA	E	3.8	16	Olive oil	76	0	NS	B	3	Un	DM II	II
	17	Purified DHA	D	3.7			72	+1	NS					
Lungershausen, 1997	16	Fish oil	ED	3.4	16	Corn oil	81	+1	NS	B	4	Un	DM I&II	II
Hendra, 1990	37	Fish oil	ED	3.0	37	Olive oil	85	-3	NS	B	4	Un	DM II	I
Jain, 2002	25	Fish oil	ED	0.6	15	"Placebo"	82	-2	.0003	C	2	Un	DM II	II

a-e See Table 3.10

among populations with type I or type II diabetes, and there were insufficient data to comment on age, sex, menopausal status, race, weight or other variables.

Dose and Source Effect

No study compared different doses of omega-3 fatty acids. Woodman et al. compared purified EPA and purified DHA and found a net fall in mean 24 hour ambulatory systolic blood pressure in subjects on EPA and a net increase in diastolic pressure; however, there was no statistical difference between the 2 treatments [120]. Across studies, there is no apparent difference in effect on systolic blood pressure based on fish oil supplement dose. However, the largest, and significant, reductions in diastolic pressure were found in the 2 studies with the smallest fish oil supplementation doses.

Exposure Duration

In 3 studies no differences in effect are noted based on duration of intervention or exposure at 3 and 6 weeks [115], 6 and 12 weeks [118], or 6 and 12 months [119].

Sustainment of Effect

No study reported blood pressures after subjects stopped treatment.

Hemoglobin A_{1c}

(Table 3.12)

Chronically elevated serum glucose levels, which occur in diabetes, result in elevated levels of glucose binding to hemoglobin. This bound product, hemoglobin A_{1c} (Hgb A_{1c}), or glycohemoglobin, is used to measure long-term control of diabetes.

We found 32 studies that met eligibility criteria and reported data on the effect of omega-3 fatty acids on Hgb A_{1c} levels (See Table 3.1). Of these, we analyzed the 18 randomized trials with data on at least 10 subjects in either parallel trials or crossover trials who consumed omega-3 fatty acids.

Overall Effect [77,85,88,93,102,103,106,115,117-126]

Across the 18 studies, omega-3 fatty acids had a very small, if any, effect on Hgb A_{1c} levels compared to control. The range of net effects across the studies was –0.4% to +1.0%. Only 1

study reported a statistically significant reduction in Hgb A_{1c}; however, this study by Jain et al. found one of the smaller net changes of all studies [117].

Sub-populations

As expected, the large majority of studies evaluating Hgb A_{1c} included diabetic patients. Fourteen studies analyzed diabetic populations, 3 of which were also dyslipidemic. An additional 2 studies analyzed dyslipidemic patients; 1 included patients with untreated hypertension; and 1 evaluated healthy monks.

While none of the 4 studies of dyslipidemic patients had net reductions in Hgb A_{1c} levels, given the small differences in almost all studies, there are no clear difference in effect in the different populations, including diabetic patients.

Covariates

Schectman et al. found that the effect of fish oil supplements on Hgb A_{1c} did not correlate with baseline differences in diet or with individual changes in diet or body weight [93]. Toft et al. and Westerveld et al. reported no change in effect of fish oil supplements on Hgb A_{1c} after adjustment for body weight [125,126]. Likewise, Haines et al reported no relationship between effect on Hgb A_{1c} and sex [115]. Three studies were notable for including only men [85,88], or because all subjects were taking simvastatin [106]. The effect found in these studies was not clearly different than that found in studies.

Table 3.12 Effects of omega-3 fatty acids on hemoglobin A_{1c} (%) in randomized trials (4 weeks to 1 year)

Author, Year	Omega-3 Fatty Acid Arm [a]				Control		Results [b]			Quality [c]				
	N	Source		g/d	N	Source	Base	Net Δ	*P*	Summary	Jadad	Allocation Conceal	Applicability [d]	
		DHA/EPA Oils												
Haines, 1986	19	Fish oil	ED	4.6	22	Olive oil	11.1	+0.2	NS	B	2	Ad	IDDM	II
Jensen, 1989	18 [e]	Cod liver oil	ED	4.6	18 [e]	Olive oil	9.5	+0.1	NS	B	4	Un	IDDM	II
Rossing, 1996	14	Cod liver oil	ED	4.6	15	Olive oil	8.8	-0.3	NS	A	3	Un	IDDM	II
Schectman, 1988	11 [e]	Fish oil	ED	4.0	11 [e]	Safflower	7.9	+0.1	NS	B	2	Un	NIDDM	II
Woodman, 2002	17	Purified EPA	E	3.8	16	Olive oil	7.1	+0.2	NS	B	3	Un	DM II	II
	18	Purified DHA	D	3.7			7.5	0	NS					
Toft, 1995	38	Fish oil	ED	3.4	40	Corn oil	5.7	+0.1	NS	A	5	Ad	CVD	II
Harris, 1997	22	Fish oil	ED	3.4	18	Corn oil	5.3	0	NS	B	3	Un	DysLip	II
Nordoy, 1998	21	Fish oil	ED	3.4	20	Corn oil	5.8	+0.2	NS	B	4	Un	DysLip	I
Lungershausen, 1997	16	Fish oil	ED	3.4	16	Corn oil	8.5	+0.2	NS	B	4	Un	DM	II
Deslypere, 1992	14	Fish oil	T	3.4	14	Olive oil	4.8	+0.2	NS	B	2	Un	GEN	III
	15	Fish oil	T	2.2			4.9	-0.1	NS					
	15	Fish oil	T	1.1			5.0	-0.1	NS					
Bonnema, 1995	14	Fish oil	ED	3.3	14	Olive oil	8.0	+1.0	NS	A	3	Ad	IDDM	II
McVeigh, 1993	23 [e]	Fish oil	ED	3.0	23 [e]	Olive oil	9.6	+0.2	NS	A	4	Un	DM II	II
Pedersen, 2003	23	Fish oil	ED	2.6	21	Corn oil	8.2	0.0	NS	A	3	Un	DysLip DM II	II
Luo, 1998	10 [e]	Fish oil	ED	1.8	10 [e]	Sunflower	8.8	-0.4	NS	B	3	Un	DM II	II
Westerveld, 1993	8	Purified EPA	E	1.8	8	Olive oil	8.2 [f]	-0.4 [g]	NS	C	3	Un	NIDDM	II

	8	Purified EPA	E	0.9			7.6	+0.4	NS					
Sirtori, 1998	203	Fish oil	ED	1.7 [h]	211	Olive oil	7.3	+0.6	NS	B	4	Ad	DysLip NIDDM	I
Jain, 2002	25	Fish oil	ED	0.6	15	"Placebo"	8.0	-0.1	.009	C	2	Un	DM II	II
		Fish and Mediterranean Diets												
Dunstan, 1997	26	Fatty Fish	T	3.6	23	No fish	8.2	+0.3	.06	B	2	Un	DysLip NIDDM	I

a A = ALA; D = DHA; E = EPA; ED = EPA+DHA; T = Total omega-3 fatty acids.
b Base = baseline level in treatment arm; Net Δ = net difference in change in omega-3 fatty acids arm compared with the change in control arm, see Methods; *P* = *P* value of difference between treatment and control arms; NS = not statistically significant.
c A = good quality; B = fair quality; C = poor quality; Jadad = Jadad Score (0-5, based on randomization, double blinding, and dropouts); Ad = adequate allocation concealment; In = inadequate allocation concealment; Un = allocation concealment unclear. See Methods.
d CVD = history of cardiovascular disease; DM I/II = diabetes mellitus type 1 or 2; DysLip = dyslipidemia; GEN = general, healthy population; N/IDDM = (non-) insulin dependent diabetes mellitus. I = broadly applicable; II = applicable to sub-group; III = narrow applicability. See Methods.
e Cross-over study.
f According to text. In table, baseline Hgb A_{1c}=8.6%
g Per data in text. Per data in table, net change = -0.8%
h 2.6 g/day for first 2 months, then 1.7 g/day for 4 months.

Dose and Source Effect

Two studies compared different doses of fish oil supplements. Deslypere et al., in a 1 year study of healthy Belgian monks, reported no difference in the effect of 3 doses of fish oil or olive oil [85]. Westerveld et al. also reported no difference in the effect of 2 different doses of fish oil, purified EPA, or olive oil in non-insulin dependent diabetics [126]. Across studies, there was no apparent dose effect of fish oil supplements. The only study of dietary fish found a lack of effect similar to the fish oil supplement studies. Woodman et al. compared purified EPA to DHA in type II diabetics [120]. No difference was noted between the 2 treatments.

Exposure Duration

Two studies reported treatment effect at multiple time points. In Haines et al. there was a transient drop in Hgb A_{1c} by 0.6% (0.5% net) at 3 weeks which reverted to baseline at 6 weeks [115]. The change was not statistically significant. Rossing et al. found no difference in effect between 6 and 12 months [119]. Across studies there was no apparent effect of treatment duration.

Sustainment of Effect

Jensen et al., in a crossover study, found that Hgb A_{1c} remained unchanged 8 weeks after stopping oil supplementation.

Fasting Blood Sugar (Table 3.13)

Elevated fasting blood sugar (FBS) is a risk factor or indicator of diabetes. People with diabetes or with altered glucose tolerance have a highly elevated risk of CVD. As discussed in the introduction, the effect of omega-3 fatty acids on diabetic control is unclear.

We found 57 studies that met eligibility criteria and reported data on the effect of omega-3 fatty acids on FBS levels (See Table 3.1). Of these, we analyzed the 17 randomized trials with data on at least 25 subjects in parallel trials and 15 subjects in crossover trials who consumed omega-3 fatty acids.

Overall Effect [52,53,68,76,77,103,116,117,120,123,125,127-132]

The effect of omega-3 fatty acids on FBS was inconsistent across the 17 studies. Four studies found large and/or near-significant net increases in FBS compared to control; 3 found large and/or significant net decreases in FBS and the rest found small non-significant changes. Across the studies, the net effect ranged between a decrease of 29 mg/dL over 8 weeks and an increase of 25 mg/dL over 6 weeks. Interpretation of the overall data is further complicated by inconsistent patterns of effect within individual study arms. In omega-3 fatty acid arms and in control arms, FBS increased from baseline in half the arms and either decreased or remained unchanged in the other half.

Sub-populations

Seven studies evaluated diabetic populations, 2 of which also had dyslipidemia; an additional 5 studies evaluated patients with dyslipidemia. Three studies included subjects who had CVD or were at increased risk for CVD (due to either diabetes or dyslipidemia). Two studies were of healthy populations.

The findings within the diabetic populations were inconsistent. The largest net decrease in FBS was found by Jensen et al. in the only study of insulin-dependent diabetics [103], while the largest net increase in FBS with omega-3 fatty acids was seen in Woodman et al. in one of the studies of type II diabetics [120]. Furthermore in each of the 3 groups of subjects on fish oil supplements in these 2 studies, FBS rose by approximately 10 or 20 mg/dL; the large difference

Table 3.13 Effects of omega-3 fatty acids on fasting blood sugar (mg/dL) in randomized trials (4 weeks to 2 years)

Author, Year	Omega-3 Fatty Acid Arm [a]				Control		Results [b]			Quality [c]			
	N	Source		g/d	N	Source	Base	Net Δ	*P*	Summary	Jadad	Allocation Conceal	Applicability [d]
		DHA/EPA Oils											
Jensen, 1989	18 [e]	Cod liver oil	ED	4.6	18 [e]	Olive oil	178	-29	NS	B	4	Un	IDDM II
Mori, 2000	19	Purified EPA	E	3.8	20	Olive oil	91	+2	.06	B	4	Un	DysLip II
	17	Purified DHA	D	3.7			92	-3	NS				
Woodman, 2002	17	Purified EPA	E	3.8	16	Olive oil	134	+25	.002	B	3	Un	DM II II
	18	Purified DHA	D	3.7			149	+18	.002				
Mackness, 1994	41	Fish oil	ED	3.4	38	Corn oil	91	+2	NS	A	3	Un	DysLip I
Toft, 1995	38	Fish oil	ED	3.4	40	Corn oil	99	+2	.06	A	5	Ad	CVD II
Grundt, 1995	28	Fish oil	ED	3.4	28	Corn oil	85	0	NS	B	2	Un	DysLip II
Eritsland, 1995b	255	Fish oil	ED	3.3	245	No oil	86	+1	NS	B	2	Ad	CVD II

Leigh-Firbank, 2002	55 [e]	Fish oil	ED	3.0	55 [e]	Olive oil	99	+3	NS	B	3	Un	DysLip	I
Hendra, 1990	37	Fish oil	ED	3.0	37	Olive oil	202	+14	NS	B	4	Un	DM II	I
McVeigh, 1993	23 [e]	Fish oil	ED	3.0	23 [e]	Olive oil	184	+7	NS	A	4	Un	DM II	II
Sirtori, 1998	203	Fish oil	ED	1.7 [f]	211	Olive oil	149	+2	NS	B	4	Ad	DysLip NIDDM	I
Jain, 2002	25	Fish oil	ED	0.6	15	"Placebo"	139	-10	.004	C	2	Un	DM II	II
		Fish and Mediterranean Diets												
Mori, 1999	17	Fatty Fish [g]	T	3.7	16	No fish [g]	95	+4 [h]	NS	B	2	Un	CVD	II
	14	Fatty Fish [i]			16	No fish [i]	94	-1 [h]	NS					
Dunstan, 1998	14	Fatty Fish [j]	T	3.6	11	No fish [j]	180	-4	NS	B	2	Un	DysLip NIDDM	I
	12	Fatty Fish [k]			12	No fish [k]	160	+5	NS					
Singh, 2002	499	Indo-Mediterranean	T	1.8	501	NCEP I [L]	108	-5	<.0001	C	2	Un	CVD risk [m]	III
		Combinations												
Freese, 1997a	16	Fish oil	ED	5.2	--	--	85	+5 [n]	nd [o]	C	3	Un	GEN	II
	22	Linseed oil	A	5.9			86	-1 [p]	nd [o]					
Finnegan, 2003	31	Fish oil margarine/ Fish oil	ED	1.7	30	Sunflower margarine	97	-3	NS	A	4	Un	DysLip	I
	30	Fish oil margarine	ED	0.8			97	-3	NS					
	30	Rapeseed/Linseed margarine	A	4.5			99	-5	NS					

nd = no data

a A = ALA; D = DHA; E = EPA; ED = EPA+DHA; T = Total omega-3 fatty acids.

b Base = baseline level in treatment arm; Net Δ = net difference in change in omega-3 fatty acids arm compared with the change in control arm, see Methods; *P* = *P* value of difference between treatment and control arms; NS = not statistically significant.

c A = good quality; B = fair quality; C = poor quality; Jadad = Jadad Score (0-5, based on randomization, double blinding, and dropouts); Ad = adequate allocation concealment; In = inadequate allocation concealment; Un = allocation concealment unclear. See Methods.

d CVD = history of cardiovascular disease; DM I/II = diabetes mellitus type 1 or 2; DysLip = dyslipidemia; GEN = general, healthy population; N/IDDM = (non-) insulin dependent diabetes mellitus. I = broadly applicable; II = applicable to sub-group; III = narrow applicability. See Methods.

e Cross-over study.

f 2.6 g/day for first 2 months, then 1.7 g/day for 4 months.

g Weight-maintaining diet.

h Estimate from graph.

i Weight-loss diet.

j Moderate exercise.

k Light exercise.

L National Cholesterol Education Program step I prudent diet.

m One or more of: hypercholesterolemia, hypertension, diabetes, angina pectoris or myocardial infarction.

n Pre-post difference (not compared to control); *P*<.05 compared to baseline.

o NS between treatments.

p Pre-post difference (not compared to control); not significantly different than baseline.

in net effect is due to the difference in effect of the olive oil control (+49 mg/dL and –7 mg/dL, respectively). In the remaining studies of diabetics, the change in FBS was in the same direction in omega-3 fatty acid arms and control arms; in 6 omega-3 study arms FBS rose from 10 mg/dL to 23 mg/dL; in 4 arms FBS fell from –2 mg/dL to –16 mg/dL. In studies of diabetics, factors other than omega-3 fatty acid consumption – such as those related to population characteristics, other treatments, or study design – appear to have had a greater effect on change in FBS than the omega-3 fatty acid treatment itself.

Among the 7 studies of dyslipidemic populations, 2 of which were also diabetic, all found a small non-significant net effect of omega-3 fatty acids on FBS that ranged from –4 to +5 mg/dL. Only Dunstan et al. found large changes in individual omega-3 fatty acid arms, which were related primarily to exercise level and were similar to the changes in the no fish control arms [127].

The 4 studies of CVD patients or people with an elevated risk of CVD all found small absolute and net changes in FBS with omega-3 fatty acid consumption. Only Singh et al. found a significant net change and had a relatively large absolute change (-8 mg/dL) in FBS, although notably about 20% of the subjects were diabetic, two-thirds were vegetarian, and those subjects on the Indo-Mediterranean diet on average lost 3 kg more weight than controls [76]. In addition, this study reported uniform, highly significant effects on all serum markers despite widely ranging effects. A number of statistical calculation errors were also found.

The single study of a healthy population, by Freese et al., found small differences in FBS with 2 different omega-3 fatty acid treatments (in opposite directions) [128].

Covariates

Schectman et al. found that changes in FBS did not correlate with baseline differences in diet or with individual changes in diet or body weight [93]. Two studies of diabetics reported data on associations between effect and other variables. Hendra et al. reported that the change in FBS was unrelated to change in weight [116]. Woodman et al. reported that the significant effect compared to olive oil was unchanged after adjusting for age, sex, and BMI [120]. In Mori, et al. (1999), a study of obese hypertensive subjects, the direction of the absolute and net changes in FBS appear related to whether subjects were on a weight-reduction diet or not (those on a weight maintaining diet had increases in FBS, while those on a weight-reduction diet had reductions in FBS); however, they reported no interaction between fish diet and weight loss on FBS [131]. No patterns across studies are evident based on reported data on covariates.

Dose and Source Effect

No study directly compared doses of the same source of omega-3 fatty acids. In comparisons of EPA and DHA, Woodman et al. reported no difference in effect on FBS [120]; however, Mori et al. (2000) reported a trend toward increasing FBS with EPA, but no change with DHA [132]. Freese et al. reported a significant increase from baseline in FBS with fish oil supplementation compared to no change with linseed oil; however the difference between the 2 treatments was reported to be non-significant [128]. In a comparison of multiple sources of omega-3 fatty acids, Finnegan et al. found no significant differences in effect between various doses of either fish oils or plant oils [53]. Across studies, there was no discernable difference in effect based on either fish oil dose or omega-3 fatty acid source among diabetic or dyslipidemic populations.

Exposure Duration

Two studies measured FBS levels at multiple time points. Hendra et al. found that FBS rose with fish oil supplements at both 3 and 6 weeks, although the net difference with control was significant only at 3 weeks [116]. In a longer study that measured FBS at 2, 4, and 6 months, Finnegan et al. found no treatment effect at any time period [53]. The heterogeneity does not appear to be related to study duration.

Sustainment of Effect

Jensen et al., in a crossover study which found that FBS rose by large amounts in both the high-dose cod liver oil and olive oil supplement arms, found that FBS fell back near baseline levels 8 weeks after stopping oil supplementation, although none of the levels were significantly different from each other [103]. Freese et al., who compared fish oil to linseed oil supplements, reported that FBS, which had risen in the fish oil arm, returned to baseline during a 12 week follow-up period [128].

Fasting Insulin

(Table 3.14)

In people with normal glucose levels (euglycemia), elevated fasting insulin levels are suggestive of insulin resistance, a precursor to type II diabetes and an independent risk factor for CVD. The value of insulin levels in those with insulin resistance, including insulin resistance related to obesity, and diabetes ("hyperglycemia"), is questionable. The effect of omega-3 fatty acids on insulin resistance and fasting insulin levels is also unclear.

We found 21 studies that met eligibility criteria and reported data on the effect of omega-3 fatty acids on fasting insulin levels (See Table 3.1). Of these, we analyzed the 15 randomized trials. All but 3 of the trials were also analyzed for data on FBS or Hgb A_{1c}.

Overall Effect [52,53,68,77,88,89,106,120,122,125,129,131-134]

Baseline levels of fasting insulin varied broadly across studies. In general, studies of non-insulin-dependent diabetics and obese subjects (under "Studies of "Hyperglycemic" Subjects") had higher mean insulin levels than dyslipidemic, hypertensive, or healthy patients (under Studies of "Euglycemic" Subjects). However, within each population grouping the range of insulin levels remained broad. Mean insulin levels varied within studies also. In 6 studies, baseline insulin levels differed between omega-3 fatty acid arms and control arms by 20% to 60%. Among these, Toft et al. reported a significant difference at baseline and Chan et al. reported no significant difference; the remaining studies did not comment [125,133]. In an attempt to standardize across studies, given the large variation in insulin levels, we calculated net differences in terms of percent change from baseline instead of absolute changes.

Table 3.14 Effects of omega-3 fatty acids on fasting insulin (pmol/L) in randomized trials (4 weeks to 9 months)

Author, Year	Omega-3 Fatty Acid Arm [a]				Control		Results [b]			Quality [c]				
	N	Source		g/d	N	Source	Base [e]	Net % Δ	*P*	Summary	Jadad	Allocation Conceal	Applicability [d]	
Studies of "Euglycemic" Subjects														
		DHA/EPA Oils												
Mori, 2000	19	Purified EPA	E	3.8	20	Olive oil	9	+28%	.04	B	4	Un	DysLip	II
	17	Purified DHA	D	3.7			10	+29%	.001					
Toft, 1995	38	Fish oil	ED	3.4	40	Corn oil	52 [f]	-1%	NS	A	5	Ad	CVD	II
Grundt, 1995	28	Fish oil	ED	3.4	28	Corn oil	66	-15%	NS	B	2	Un	DysLip	II
Nordoy, 1998	21	Fish oil	ED	3.4	20	Corn oil	12 [g]	-28%	NS	B	4	Un	DysLip	I
Eritsland, 1995b	255	Fish oil	ED	3.3	245	No oil	125	-1%	NS	B	2	Ad	CVD	II
Leigh-Firbank, 2002	55 [h]	Fish oil	ED	3.0	55 [h]	Olive oil	72	-1%	NS	B	3	Un	DysLip	I
Marckmann, 1997	23	Fish oil margarine	T	0.9	24	Sunflower margarine	64 [i]	-8%	NS	B	3	Un	GEN	II
		Fish and Mediterranean Diets												
Mori, 1999	17	Fatty fish [j]	T	3.7	16	No fish [j]	12	+14%	NS	B	2	Un	CVD [k]	II
	14	Fatty fish [L]			16	No fish [L]	13	-18%	NS					
		Combinations												
Finnegan, 2003	31	Fish oil margarine/ Fish oil	ED	1.7	30	Sunflower margarine	42	0%	NS	A	4	Un	DysLip	I
	30	Fish oil margarine	ED	0.8			57 [m]	-16%	NS					
	30	Rapeseed/Linseed margarine	A	4.5			49 [m]	-19%	NS					
Studies of "Hyperglycemic" Subjects														
		DHA/EPA Oils												
Woodman, 2002	17	Purified EPA	E	3.8	16	Olive oil	98	+4%	NS	B	3	Un	DM II	II
	18	Purified DHA	D	3.7			115	+3%	NS					
Chan, 2003	12	Fish oil	ED	3.1	12	Corn oil	285 [n]	+12%	NS	A	4	Un	GEN [o]	III
Rivellese, 1996	8	Fish oil	ED	2.6 [p]	8	Olive oil	75 [q]	+29%	NS	A	3	Un	DysLip NIDDM	II
Luo, 1998	10 [h]	Fish oil	ED	1.8	10 [h]	Sunflower	84	+15%	NS	B	3	Un	DM II	II
Sirtori, 1998	203	Fish oil	ED	1.7 [r]	211	Olive oil	116	-11%	NS	B	4	Ad	DysLip NIDDM	I
		Fish and Mediterranean Diets												
Dunstan, 1997	14	Fatty fish [s]	T	3.6	11	No fish [s]	78	-25% [t]	.08	B	2	Un	DysLip NIDDM	I
	12	Fatty fish [u]			12	No fish [u]	78	-28% [t]	.05					

a A = ALA; D = DHA; E = EPA; ED = EPA+DHA; T = Total omega-3 fatty acids.

b Base = baseline level in treatment arm; Net % Δ = net percent difference in change in omega-3 fatty acids arm compared with the change in control arm, see Methods; *P* = *P* value of difference between treatment and control arms; NS = not statistically significant.

c A = good quality; B = fair quality; C = poor quality; Jadad = Jadad Score (0-5, based on randomization, double blinding, and dropouts); Ad = adequate allocation concealment; In = inadequate allocation concealment; Un = allocation concealment unclear. See Methods.

d CVD = history of cardiovascular disease; DM I/II = diabetes mellitus type 1 or 2; DysLip = dyslipidemia; GEN = general, healthy population; N/IDDM = (non-) insulin dependent diabetes mellitus. I = broadly applicable; II = applicable to sub-group; III = narrow applicability. See Methods.

e Studies with a greater than 20% difference between treatment and control are noted.
f Mean insulin in control arm = 64 pmol/L. Reported to be significantly different than treatment arm.
g Mean insulin in control arm = 9 pmol/L. No data on whether significantly different than treatment arm.
h Cross-over study.
i Mean insulin in control arm = 53 pmol/L. No data on whether significantly different than treatment arm.
j Weight-maintaining diet.
k Overweight.
L Weight-loss diet.
m Mean insulin in control arm = 37 pmol/L. No data on whether significantly different than treatment arm.
n Mean insulin in control arm = 215 pmol/L. Reported as not significantly different from treatment arm.
o Obese men.
p 2.6 g/day for first 2 months, then 1.7 g/day for 1 month.
q Mean insulin in control arm = 121 pmol/L. No data on whether significantly different than treatment arm.
r 2.6 g/day for first 2 months, then 1.7 g/day for 4 months.
s Moderate exercise.
t Percent decrease based on baseline level in fish diet arm, derived from regression analysis.
u Light exercise.

Across the 15 studies there were a wide range of apparent treatment effects ranging from net changes of –28% to +29% (or –22 pmol/L in Dunstan et al. [122] to +34 pmol/L in Chan et al. [133]). Approximately one-third of the omega-3 fatty acid study arms had net percent changes of either greater than +10%, between –10% and +10%, or less than –10%.

Sub-populations

Nine of the studies reported data on essentially euglycemic populations. The remaining 6 studies evaluated diabetic or obese populations in which the fasting insulin level may be of less value. While the studies with hyperglycemic subjects all had elevated mean fasting insulin levels, there was a wide range of mean insulin levels in the studies of euglycemic subjects.

Among the studies of euglycemic subjects, the heterogeneity of effect was similar to the heterogeneity seen across all studies. The heterogeneity was particularly apparent among the studies of dyslipidemic patients.

Covariates

Among the studies of euglycemic subjects, Mori et al. (1999) reported no interaction between dietary fish intake and weight loss on insulin levels [131]. However, a weight loss diet resulted in a reduction of insulin levels, regardless of fish consumption. In addition, there was a net decrease in insulin levels in subjects who were on a weight loss diet with fish compared to a net increase in insulin in subjects who were on a weight-maintaining diet. Otherwise, studies did not attempt to correlate the effect on insulin of covariates. The 3 studies that either included only euglycemic men [89,132] or excluded pre-menopausal women [131] had a wide range of effects on insulin levels. Thus, no potential sex effect could be seen.

No study of hyperglycemic subjects reported a correlation between insulin and covariates. As in studies of euglycemic subjects the effects on insulin found among the 2 studies of hyperglycemic men [88,133] and the study that excluded pre-menopausal women [120] were heterogeneous.

Dose and Source Effect

Finnegan et al. compared plant oil margarine to 2 doses of fish oil (as margarine and as both margarine and supplement) and to omega-6 fatty acid margarine [53]. None of the differences in insulin levels was statistically significant and the article does not comment on the relative effects of different treatments. However, dyslipidemic subjects on ALA margarine had an absolute and net decrease in fasting insulin, while subjects on low dose fish oil had a small absolute increase in insulin that was less than the increase in the control group, and subjects on high dose fish oil had an increase in insulin similar to controls. Across the studies, the effect on insulin does not appear to be associated with fish oil dose.

Both Mori et al. (2000) and Woodman et al. compared purified EPA to DHA, although in different populations [120,132]. No difference was noted between the 2 treatments in both studies.

Exposure Duration and Sustainment of Effect

Only Finnegan et al. measured insulin levels at multiple time points [53]. They reported no treatment-time interaction with insulin levels at 2, 4, and 6 months. No study measured insulin levels after ceasing omega-3 fatty acid consumption.

C-Reactive Protein

(Table 3.15)

C-reactive protein (CRP) is an acute phase reactant produced in the liver. It is thought to represent an integrated assessment of the overall state of activation of the inflammatory system. Recently, a high sensitivity assay for measuring CRP has been developed that can detect levels of CRP below what was previously considered the 'normal' range. A growing body of studies suggest that elevations in CRP levels detected by the high sensitivity assay predict a poor cardiovascular prognosis [135].

All eligible studies that reported on the effect of omega-3 fatty acids on CRP levels were included; 5 studies qualified. Four were randomized trials of oil supplements or diet; 1 was a retrospective cross-sectional analysis of usual diet.

Overall Effect [56,99,136-138]

No study found a significant effect of omega-3 fatty acid consumption on CRP level. However, CRP levels increased relative to subjects who were on control oils in most study arms among the 4 randomized trials. In contrast, the cross-sectional study did find that CRP levels were lower among subjects who ate fish regularly (fish score >4) but the difference was not statistically significant.

Sub-populations and Covariates

No study directly compared the effect of omega-3 fatty acids with placebo in different populations. There was no clear difference in effect across studies based on population. Baseline CRP levels varied across studies; although the reason for the different CRP levels is not apparent. Madsen et al. reported that when the 11 subjects with baseline CRP greater than 2 mg/L were analyzed separately, no difference in effect was seen with fish oil supplementation (as in all subjects) [137]. Likewise, the effect of omega-3 fatty acids does not appear to differ across studies based on average baseline CRP.

The trial by Chan et al. was a factorial study with fish oil supplements and atorvastatin (40 mg/day) in obese men who had a substantially higher baseline CRP than a separate group of 10 lean men (0.49 mg/L) [139]. While atorvastatin did significantly reduce CRP levels (by 0.73 mg/L) there was no interaction with fish oil.

Table 3.15 Effects of omega-3 fatty acids on C-reactive protein (mg/L) in studies (4 wk to 3 mo or cross-sectional)

Author, Year	Omega-3 Fatty Acid Arm [a]				Control		Results [b]			Quality [c]				
	N	Source		g/d	N	Source	Base	Net Δ	*P*	Summary	Jadad	Allocation Conceal	Applicability [d]	
RCTs														
		DHA/EPA Oils												
Madsen, 2003	20	Fish oil	ED	5.9	20	Olive oil	[1.07]	[-0.15]	NS	B	3	Un	GEN	I
	20	Fish oil	ED	1.7			[0.69]	[+0.02]	NS					
Chan, 2002	12	Fish oil	ED	3.4	12	Corn oil	2.11	+0.05	NS [e]	B	3	Un	DysLip	II
		Plant oils												
Junker, 2001	18	Rapeseed oil diet	T	2.5% [f]	40	Olive or Sunflower	0.5 [g]	+0.11 [h]	NS	C	1	Un	GEN	I
		Fish and Mediterranean Diets												
Mezzano, 2001	21	Mediterranean	T	1.6	21	Red meat	4.9	+1.7	NS	C	1	In	GEN	III
Cross-Sectional								**Cohort Δ**						
		Diets												
Madsen, 2001	43	Fish Score 5-6			24	Fish Score 2-4	2.3	-0.1 [i]	NS	--	--	--	CVD	II
	83	Fish Score 7-8					1.9	-0.5 [i]						
	102	Fish Score 9-10					2.1	-0.3 [i]						
	127	Fish Score 11-12					2.2	-0.1 [i]						

a A = ALA; D = DHA; E = EPA; ED = EPA+DHA; T = Total omega-3 fatty acids.

b Base = baseline level in treatment arm (numbers in square brackets are median values or net differences of median values); Net Δ = net difference in change in omega-3 fatty acids arm compared with the change in control arm, see Methods; Cohort Δ = difference in CRP between cohort and reference cohort (cross-sectional); *P* = *P* value of difference between treatment and control arms; NS = not statistically significant.

c A = good quality; B = fair quality; C = poor quality; Jadad = Jadad Score (0-5, based on randomization, double blinding, and dropouts); Ad = adequate allocation concealment; In = inadequate allocation concealment; Un = allocation concealment unclear. See Methods.

d CVD = history of cardiovascular disease; DM I/II = diabetes mellitus type 1 or 2; DysLip = dyslipidemia; GEN = general, healthy population; N/IDDM = (non-) insulin dependent diabetes mellitus. I = broadly applicable; II = applicable to sub-group; III = narrow applicability. See Methods.

e Statistical significance based on 23 subjects on Omacor and 25 on placebo, half of whom were also on atorvastatin.

f Kcal.
g Median.
h Net difference of median values of rapeseed compared to average change in 2 control groups.
i Difference between cohort and low-fish cohort (fish score 2-4). Back-calculated from reported ln(CRP).

Dose and Source Effect

No study compared different sources of omega-3 fatty acids. Any differences in effect due to differing sources across studies could not be appreciated among the few studies. The cross-sectional study did not find an association between fish score (amount of fish in diet) and CRP level.

Exposure Duration

Junker et al. evaluated CRP levels at both 2 and 4 weeks. No differences were noted between baseline and either 2 or 4 weeks [56]. Mezzano et al. evaluated CRP levels at 30 days and 90 days (and also at 60 days after 30 days of added red wine). CRP was unchanged at all observation points.

Sustainment of Effect

No study re-examined CRP after subjects stopped taking omega-3 fatty acids.

Fibrinogen

(Table 3.16)

Fibrinogen, a liver protein necessary for clotting, has been found to be both increased in patients with ischemic heart disease and a predictor of cardiovascular events. It is unknown whether reducing fibrinogen levels would alter cardiovascular risk. In addition, there is currently no standardized measurement technique.

We found 59 studies that met eligibility criteria and reported data on the effect of omega-3 fatty acids on fibrinogen levels (See Table 3.1). Of these, we analyzed the 24 randomized trials with data on at least 15 subjects in parallel trials and 10 subjects in crossover trials who consumed omega-3 fatty acids.

Overall Effect [46,56,69,74,85,89,90,100,115,116,138,140-152]

Across the 24 studies there was no consistent effect on fibrinogen levels of omega-3 fatty acid consumption compared to control. Approximately half the omega-3 fatty acid study arms resulted in a net increase in fibrinogen level compared to control; in the other half there was either a net decrease or no effect on fibrinogen level. Only 4 studies reported a statistically significant difference between the effect of omega-3 fatty acid and control. In 3 of these, the net decrease of fibrinogen ranged from approximately 5% to 20%. One study reported a significant net increase of fibrinogen of 11%.

Sub-populations

Thirteen of the studies evaluated generally healthy subjects. No consistent effect was found specifically in this population. Four studies evaluated subjects with known CVD: 2 studies of patients with stable claudication (Gans et al. and Leng et al.) [69,144], one of patients who were undergoing coronary bypass (Eritsland et al.) [142], and one of subjects with hypertension (Toft et al.) [152]. All 4 studies found no effect of omega-3 fatty acids on fibrinogen levels. Seven studies included subjects with diabetes and/or dyslipidemia. Again, there was no consistent effect. However, the largest (significant) net decrease in fibrinogen was found by Radack et al. in a group of 10 subjects with hyperlipoproteinemia types IIb or IV on a moderate dose of fish oil supplement [151]. A significant net increase in fibrinogen was seen by Haines et al. among 19 subjects with insulin-dependent diabetes on a high dose of fish oil supplement, although the effect was not related to Hgb A1c level. [115].

In the study of patients undergoing coronary bypass, Eritsland et al. found that the (lack of) effect of omega-3 fatty acids on fibrinogen was unchanged after adjusting for multiple factors including age and sex [142]. Seven studies included only men [46,85,100,138,140,147,149]. The distribution of effects was similar in this subset of studies as in the whole set. Three of these studies of men and an one additional study included only younger adults (generally less than 30 or 40 years old) [46,138,140,146]. These studies had results similar to studies of broader age ranges or of older subjects. Overall, the studies provided insufficient data on race or ethnicity to allow analysis of these subpopulations. Almost half the studies were performed in Scandinavia and Finland; most of the remaining are from northern Europe and Australia. Notably the study by Radack et al., which

Table 3.16 Effects of omega-3 fatty acids on fibrinogen (g/L) in randomized trials (4 weeks to 2 years)

Author, Year	Omega-3 Fatty Acid Arm [a]				Control		Results [b]			Quality [c]				
	N	Source		g/d	N	Source	Base	Net Δ	*P*	Summary	Jadad	Allocation Conceal	Applicability [d]	
		DHA/EPA Oils												
Hansen, 1989	40 [e]	Cod liver oil	ED	5.8	40 [e]	No oil	2.4	-0.1	NS	C	1	Un	GEN	I
Haines, 1986	19	Fish oil	ED	4.6	22	Olive oil	2.7	+0.3	<.05	B	2	Ad	IDDM	II
Misso, 1995	12 [e]	Fish oil	ED	3.6	12 [e]	Olive oil	3.0	+0.2	NS	C	2	Un	GEN	II
Hansen, 1993a	11	Fish oil Tg [f]	ED	3.6	10	Corn oil	2.4	+0.3 [g]	nd	B	4	Un	GEN	II
	10	Fish oil EE [h]	ED	3.4			2.4	-0.1 [g]	nd					
Toft, 1997	38	Fish oil	ED	3.4	38	Corn oil	2.2	+0.2	NS	A	5	Ad	CVD	II
Grundt, 1999	28	Ethyl ester [i]	ED	3.4	28	Corn oil	2.9	-0.1	NS	B	2	Un	DysLip	II
Nordoy, 2000	21	Fish oil	ED	3.4	20	Corn oil	3.0	+0.1	NS	B	4	Un	DysLip	I
Deslypere, 1992	14	Fish oil	T	3.4	14	Olive oil	2.3	-0.1	NS	B	2	Un	GEN	III
	15	Fish oil	T	2.2			2.3	-0.3	NS					
	15	Fish oil	T	1.1			2.0	+0.1	NS					
Eritsland, 1995c	254	Fish oil	ED	3.3	249	No oil	2.6	-0.1	NS	B	2	Ad	CVD	II
Osterud, 1995	26	Cod liver oil	ED	3.1	28	No oil	2.6	0.0	NS	B	2	Un	GEN	I
	27	Seal\Cod oil	ED	2.8			2.5	+0.1	NS					
	27	Seal oil	ED	2.4			2.6	0.0	NS					
	26	Whale oil	ED	1.7			2.6	-0.1	NS					
Hendra, 1990	37	Fish oil	ED	3.0	37	Olive oil	3.2	+0.2	NS	B	4	Un	DM II	I
Gans, 1990	16	Fish oil	ED	3.0	16	Corn oil	3.3	+0.1	NS	A	3	Ad	CVD	II

Radack, 1989	10	Fish oil	ED	2.2	8	Olive oil	3.2	-0.6 [j]	<.05	B	3	Un	DysLip	II
	7	Fish oil	ED	1.1			2.9	0.0	NS					
Marckmann, 1997	23	Fish oil margarine	T	0.9	24	Sunflower margarine	2.4	-0.05	NS	B	3	Un	GEN	II
Nenseter, 2000	34	Fish powder	ED	0.2	36	Cellulose	3.0	-0.2	NS	B	3	Un	DysLip	I
Leng, 1998	37 [k]	Fish oil	ED	0.045 [L]	36 [m]	Sunflower oil	3.4	+0.04	NS	C	4	Ad	CVD	II
		Plant Oils												
Allman-Farinelli, 1999	15	Flaxseed oil diet	A	10% [n]	14	Safflower oil	2.1	+0.1	NS	B	2	Un	GEN	II
Junker, 2001	18	Rapeseed oil	T	2.5% [o]	40	Olive or Sunflower oil	2.3	+0.1 [p]	NS	C	1	Un	GEN	I
		Fish and Mediterranean Diets												
Muller, 1989	40	Mackerel paste	ED	4.7	42	Meat paste	2.7	-0.02	NS	B	1	Un	GEN	II
Dunstan, 1999	14	Fatty fish [q]	T	3.6	23	No fish	2.9	+0.2 [r]	NS	B	2	Un	DysLip NIDDM	I
	12	Fatty fish [s]					3.3	+0.1 [r]	NS					
Mezzano, 2001	21	Mediterranean	T	1.6	21	Red meat	2.3	-0.3	.03	C	1	In	GEN	III
		Combinations												
Freese, 1997b	24	Fish oil	ED	5.2	--	--	3.1	-0.06 [t]	nd [u]	C	3	Un	GEN	II
	22	Linseed oil	A	5.9			3.1	+0.05 [t]	nd [u]					
Cobiac, 1991	13	Fish oil	ED	4.6	6	Olive, Palm, Safflower oil	2.35	+0.4	NS	B	2	Un	GEN	II
	12	Fatty fish diet	ED	4.5			2.65	-0.15	<.05					
Agren, 1997	14	Fish oil	ED	2.3	14	No oil	3.6	+0.3	NS	B	3	Un	GEN	III
	14	Algae DHA oil	D	1.7			3.4	+0.1	NS					
	13	Fatty fish diet	ED	1.1			3.4	+0.3	NS					

nd = no data

a A = ALA; D = DHA; E = EPA; ED = EPA+DHA; T = Total omega-3 fatty acids.

b Base = baseline level in treatment arm; Net Δ = net difference in change in omega-3 fatty acids arm compared with the change in control arm, see Methods; *P* = *P* value of difference between treatment and control arms; NS = not statistically significant.

c A = good quality; B = fair quality; C = poor quality; Jadad = Jadad Score (0-5, based on randomization, double blinding, and dropouts); Ad = adequate allocation concealment; In = inadequate allocation concealment; Un = allocation concealment unclear. See Methods.

d CVD = history of cardiovascular disease; DM I/II = diabetes mellitus type 1 or 2; DysLip = dyslipidemia; GEN = general, healthy population; N/IDDM = (non-) insulin dependent diabetes mellitus. I = broadly applicable; II = applicable to sub-group; III = narrow applicability. See Methods.

e Cross-over study.

f Triglycerides.

g *P*=.09 between treatments.

h Ethyl esters.

i No data on source.

j *P*<.05 compared to 1.1 g/day.

k Baseline data based on N=52.

L Plus 280 mg gamma linolenic acid (omega-6 fatty acid).

m Baseline data based on N=50.

n ALA = 10% of daily fatty acid intake.

o Kcal.

p Difference compared to average change in 2 control groups.

q Moderate exercise.

r Estimate from graph. Not clear which control group compared to (or combined). Possibly adjusted for age and sex.

s Light exercise.

t Pre-post difference (not compared to control).

u NS between treatments.

showed the largest benefit from omega-3 fatty acids and was the only study to show a dose effect (see below), was the only study performed in the United States [151].

Covariates

Eritsland et al., Haines et al. and Toft et al. found no association of effect of omega-3 fatty acids on fibrinogen with various factors including sex, baseline and change in weight, baseline blood pressure, change in lipids or insulin, or cardiovascular, lipid or antithrombotic drug use among patients with cardiovascular disease [115,142,152]. Mezzano et al. found no interaction of wine consumption with a Mediterranean diet in a multiphase trial [138]. No differences were found among studies with run-in phases of either high- or low-fat diets. No study quantified baseline fish consumption. Radack et al. reported that the relative effect of higher dose fish oil supplements was greater with higher baseline fibrinogen values ($r = -0.59$, $P < .01$) [151].

Dose and Source Effect

Two studies compared different doses of the same omega-3 fatty acid supplements. Radack et al. found that subjects with dyslipidemia who took 6 g of fish oil supplements (2.2 g EPA+DHA) for 20 weeks had a relatively large, statistically significant net reduction in fibrinogen [151]. This effect was significantly greater than in the subjects who took 3 g of fish oil (1.1 g EPA+DHA), who had no effect. Deslypere et al., however, found no difference in effect across 3 doses of fish oil supplements (3.4 g, 2.2 g, and 1.1 g EPA+DHA) in monks who took fish oils for 1 year. Across all studies the effect is not related to omega-3 fatty acid dosage.

Hansen et al. (1993a) reported a possible trend toward greater effect of fish oil ethyl esters than fish oil triglycerides [147]. Osterud et al. found no difference among different marine oils [74]. Two studies evaluated ALA oils. Both found no effect with dietary flaxseed oil or rapeseed oil supplements [46,56].

Three studies compared fish oil supplements with other sources of omega-3 fatty acids [100,140,143]. Cobiac et al. found a small significant reduction in fibrinogen only among the subjects consuming dietary fish; however the significance of the difference between the 2 treatments was not reported [100]. Overall, there were no clear differences in effect of different sources of omega-3 fatty acids.

Exposure Duration

Across studies, there was no apparent effect on fibrinogen of duration of consumption of omega-3 fatty acids in studies that reported data from 2 weeks to 2 years. Seven studies reported fibrinogen levels at various time points [56,69,85,115,138,149,151]. Although mean fibrinogen levels varied with time in most studies, no study found a difference in effect related to time.

Sustainment of Effect

Two studies, which both found no effect of omega-3 fatty acids on fibrinogen levels, reported no further change after stopping treatment. Deslypere et al. reported no difference in fibrinogen levels up to 6 months after 1 year of treatment [85]. Freese et al. likewise found no difference 4 weeks after finishing 4 weeks of treatment [143].

Factor VII, Factor VIII, and von Willebrand Factor

(Tables 3.17, 3.18, and 3.19)

Omega-3 fatty acids affect the clotting system in a number of ways in animal and *in vitro* models. Factors VII and VIII and von Willebrand factor (vWF) are factors in the extrinsic coagulation system that have been suggested to play a crucial role in the initiation of blood coagulation in atherosclerotic disease, particularly in diabetes [153]. Although the mechanism is not well-established, high vWF levels help to predict cardiovascular events, although the vWF level is not powerfully predictive in the individual at risk [154]. However, different laboratories use different methods to measure coagulation factors including antigen or activity level, percent compared to a standard or concentration, and other variations. This makes comparisons across studies difficult.

We found 44 studies that met eligibility criteria and reported data on the effect of omega-3 fatty acids on factor VII, factor VIII, and/or vWF (40, 13, and 20 studies, respectively; See Table 3.1). Of these, we analyzed the 23 randomized trials that met additional criteria. For factor VII, we analyzed studies that had data on at least 15 subjects in parallel trials or 10 subjects in crossover trials who consumed omega-3 fatty acids (19 studies). For factor VIII and vWF, we analyzed all randomized trials (5 and 9 studies, respectively).

Overall Effect

Factor VII (Table 3.17) [46,56,74,89,90,115,116,138,140-143,145-147,149,150,152,155]**.** There is little consistency in effect across the 19 studies of factor VII activity. In general, the net change in factor VII in subjects consuming omega-3 fatty acids is small (7% change from baseline or less), although a nearly equal number of studies found net increases as found net decreases in levels.

Table 3.17 Effects of omega-3 fatty acids on factor VII activity (%) in randomized trials (4 weeks to 9 months)

Author, Year	Omega-3 Fatty Acid Arm [a]				Control		Results [b]			Quality [c]				
	N	Source		g/d	N	Source	Base	Net Δ	*P*	Summary	Jadad	Allocation Conceal	Applicability [d]	
		DHA/EPA Oils												
Hansen, 1989	40 [e]	Cod liver oil	ED	5.8	40 [e]	No oil	90	+2	NS	C	1	Un	GEN	I
Haines, 1986	19	Fish oil	ED	4.6	22	Olive oil	79	+5	NS	B	2	Ad	IDDM	II
Hansen, 1993a	11	Fish oil Tg [f]	ED	3.6	10	Corn oil	87	-1	NS	B	4	Un	GEN	II
	10	Fish oil EE [g]	ED	3.4			83	-2	NS					
Toft, 1997	38	Fish oil	ED	3.4	38	Corn oil	105	+1	NS	A	5	Ad	CVD	II
Grundt, 1999	28	Ethyl ester [h]	ED	3.4	28	Corn oil	119	-5	NS	B	2	Un	DysLip	II
Nordoy, 2000	21	Fish oil	ED	3.4	20	Corn oil	132	-2	NS	B	4	Un	DysLip	I
Eritsland, 1995c	90	Fish oil	ED	3.3	107	No oil	109	-6	NS	B	2	Ad	CVD	II
Osterud, 1995	26	Cod liver oil	ED	3.1	28	No oil	1.16 [i]	0	NS	B	2	Un	GEN	I
	27	Seal/Cod oil	ED	2.8			1.21 [i]	+0.03	NS					
	27	Seal oil	ED	2.4			1.23 [i]	-0.08	NS					
	26	Whale oil	ED	1.7			1.20 [i]	-0.01	NS					
Hendra, 1990	37	Fish oil	ED	3.0	37	Olive oil	94	+22	.02	B	4	Un	DM II	I
Berrettini, 1996	20	Fish oil	ED	2.6	19	Corn oil	116	0 [j]	NS	B	3	Un	CVD	II

Marckmann, 1997	23	Fish oil margarine	T	0.9	24	Sunflower margarine	104	0	NS	B	3	Un	GEN	II
Nenseter, 2000	34	Fish powder	ED	0.2	36	Cellulose	121	+1	NS	B	3	Un	DysLip	I
		Plant Oils												
Allman-Farinelli, 1999	15	Flaxseed oil diet	A	10% [k]	14	Safflower oil	83	+3 [j]	NS	B	2	Un	GEN	II
Junker, 2001	18	Rapeseed oil	T	2.5% [L]	40	Olive or Sunflower oil	101	+4 [m]	NS	C	1	Un	GEN	I
		Fish and Mediterranean Diets												
Muller, 1989	40	Mackerel paste	ED	4.7	42	Meat paste	99	-0.5	NS	B	1	Un	GEN	II
Dunstan, 1999	14	Fatty fish [n]	T	3.6	23	No fish	112	+1 [o]	NS	B	2	Un	DysLip NIDDM	I
	12	Fatty fish [p]					113	+5 [o]	<.05					
Mezzano, 2001	21	Mediterranean	T	1.6	21	Red meat	78	-4	.03	C	1	In	GEN	III
		Combinations												
Freese, 1997b	24	Fish oil	ED	5.2	--	--	89	+6 [q]	nd [r]	C	3	Un	GEN	II
	22	Linseed oil	A	5.9			90	+5 [q]	nd [r]					
Agren, 1997	14	Fish oil	ED	2.3	14	No oil	93	0	NS	B	3	Un	GEN	III
	14	Algae DHA oil	D	1.7			98	-6	NS					
	13	Fatty Fish diet	ED	1.1			94	-2	NS					

nd = no data

a A = ALA; D = DHA; E = EPA; ED = EPA+DHA; T = Total omega-3 fatty acids.

b Base = baseline level in treatment arm; Net Δ = net difference in change in omega-3 fatty acids arm compared with the change in control arm, see Methods; *P* = *P* value of difference between treatment and control arms; NS = not statistically significant.

c A = good quality; B = fair quality; C = poor quality; Jadad = Jadad Score (0-5, based on randomization, double blinding, and dropouts); Ad = adequate allocation concealment; In = inadequate allocation concealment; Un = allocation concealment unclear. See Methods.

d CVD = history of cardiovascular disease; DM I/II = diabetes mellitus type 1 or 2; DysLip = dyslipidemia; GEN = general, healthy population; N/IDDM = (non-) insulin dependent diabetes mellitus. I = broadly applicable; II = applicable to sub-group; III = narrow applicability. See Methods.

e Cross-over study.

f Triglycerides.

g Ethyl esters.

h No data on source.

i Factor VIIc activity in U/mL.

j Estimated from graph.

k ALA = 10% of daily fatty acid intake.

L Kcal.

m Difference compared to average change in 2 control groups.

n Moderate exercise.

o Estimate from graph. Not clear which control group compared to (or combined). Possibly adjusted for age and sex.

p Light exercise.

q Pre-post difference (not compared to control).

r NS between treatments.

Factor VIII (Table 3.18) [46,84,85,115,138]**.** Five studies reported data on factor VIII activity. (It is unclear whether Conquer et al. measured factor VIII activity or antigen [84].) There is no consistent effect across studies, with some finding a net increase and some a net decrease in factor VIII level.

Table 3.18 Effects of omega-3 fatty acids on factor VIII activity (%) in randomized trials (6 weeks to 1 year)

Author, Year	Omega-3 Fatty Acid Arm [a]				Control		Results [b]			Quality [c]				
	N	Source		g/d	N	Source	Base	Net Δ	*P*	Summary	Jadad	Allocation Conceal	Applicability [d]	
		DHA/EPA Oils												
Haines, 1986	19	Fish oil	ED	4.6	22	Olive oil	123	+8	NS	B	2	Ad	IDDM	II
Deslypere, 1992	14	Fish oil	T	3.4	14	Olive oil	77	-1	NS	B	2	Un	GEN	III
	15	Fish oil	T	2.2			73	-2	NS					
	15	Fish oil	T	1.1			81	+4	NS					
Conquer, 1999	9	Seal oil	ED	3.0	10	Evening primrose	0.85 [e]	+0.12	NS	A	4	Un	GEN	II
		Plant Oils												
Allman-Farinelli, 1999	15	Flaxseed oil diet	A	10% [f]	14	Safflower oil	82	-5 [g]	NS	B	2	Un	GEN	II
		Fish and Mediterranean Diets												
Mezzano, 2001	21	Mediterranean	T	1.6	21	Red meat	68	-5	.006	C	1	In	GEN	III

a A = ALA; D = DHA; E = EPA; ED = EPA+DHA; T = Total omega-3 fatty acids.
b Base = baseline level in treatment arm; Net Δ = net difference in change in omega-3 fatty acids arm compared with the change in control arm, see Methods; *P* = *P* value of difference between treatment and control arms; NS = not statistically significant.
c A = good quality; B = fair quality; C = poor quality; Jadad = Jadad Score (0-5, based on randomization, double blinding, and dropouts); Ad = adequate allocation concealment; In = inadequate allocation concealment; Un = allocation concealment unclear. See Methods.
d CVD = history of cardiovascular disease; DM I/II = diabetes mellitus type 1 or 2; DysLip = dyslipidemia; GEN = general, healthy population; N/IDDM = (non-) insulin dependent diabetes mellitus. I = broadly applicable; II = applicable to sub-group; III = narrow applicability. See Methods.
e Factor VIII in U/mL (unclear whether activity or antigen).
f ALA = 10% of daily fatty acid intake.
g Estimated from graph.

von Willebrand Factor (Table 3.19) [46,69,84,85,89,147,149,150,156]**.** Nine studies reported data on various measurements of vWF using different measurement methods. Some studies were not explicit about the specific measurement used. Most studies found a net decrease in vWF level (of up to a 13% reduction from baseline), although in only 1 study was the difference with placebo reported to be statistically significant.

Table 3.19 Effects of omega-3 fatty acids on von Willebrand factor in randomized trials (4 weeks to 2 years)

Author, Year	Omega-3 Fatty Acid Arm [a]				Control		Results [b]				Quality [c]				
	N	Source		g/d	N	Source	Base	Net Δ	Unit	*P*	Summary	Jadad	Allocation Conceal	Applicability [d]	
		DHA/EPA Oils													
Seljeflot, 1998	22	Fish oil	ED	4.8	19	Fatty acids	127	-17	% [e]	.03	B	4	Un	DysLip	II
Hansen, 1993a	11	Fish oil Tg [f]	ED	3.6	10	Corn oil	100	-13	% [g]	nd	B	4	Un	GEN	II
	10	Fish oil EE [h]	ED	3.4			121	-16		nd					
Nordoy, 2000	21	Fish oil	ED	3.4	20	Corn oil	101	-5	% [i]	NS	B	4	Un	DysLip	I
Deslypere,	14	Fish oil	T	3.4	14	Olive oil	133	-1		NS	B	2	Un	GEN	III

1992	15	Fish oil	T	2.2			141	-2	% [j]	NS					
	15	Fish oil	T	1.1			137	+7		NS					
Conquer, 1999	9	Seal oil	ED	3.0	10	Evening primrose	6.9	-0.5	µg/mL [k]	NS	A	4	Un	GEN	II
Marckmann, 1997	22	Fish oil margarine	T	0.9	24	Sunflower margarine	86	-6	% [e]	NS	B	3	Un	GEN	II
Leng, 1998	37 [L]	Fish oil	ED	0.045 [m]	36 [n]	Sunflower oil	118	+7	IU/dL [o]	NS	C	4	Ad	CVD	II
		Plant Oils													
Allman-Farinelli, 1999	15	Flaxseed oil diet	A	10% [p]	14	Safflower oil	96	-6 [q]	% [r]	NS	B	2	Un	GEN	II
		Fish and Mediterranean Diets													
Muller, 1989	40	Mackerel paste	ED	4.7	42	Meat paste	1.02	0	IU [e]	NS	B	1	Un	GEN	II

nd = no data

a A = ALA; D = DHA; E = EPA; ED = EPA+DHA; T = Total omega-3 fatty acids.

b Base = baseline level in treatment arm; Net Δ = net difference in change in omega-3 fatty acids arm compared with the change in control arm, see Methods; *P* = *P* value of difference between treatment and control arms; NS = not statistically significant.

c A = good quality; B = fair quality; C = poor quality; Jadad = Jadad Score (0-5, based on randomization, double blinding, and dropouts); Ad = adequate allocation concealment; In = inadequate allocation concealment; Un = allocation concealment unclear. See Methods.

d CVD = history of cardiovascular disease; DM I/II = diabetes mellitus type 1 or 2; DysLip = dyslipidemia; GEN = general, healthy population; N/IDDM = (non-) insulin dependent diabetes mellitus. I = broadly applicable; II = applicable to sub-group; III = narrow applicability. See Methods.

e By enzyme-linked immunosorbent assay (ELISA).

f Triglycerides.

g Factor VIII-related antigen, by ELISA.

h Ethyl esters.

i Activity.

j Plasma content, by ELISA.

k By rocket immunoelectrophoretic procedure.

L Baseline data based on N=52.

m Plus 280 mg gamma linolenic acid (omega-6 fatty acid).

n Baseline data based on N=50.

o Concentration, by ELISA.

p ALA = 10% of daily fatty acid intake.

q Estimated from graph.

r Antigen, by ELISA.

Sub-populations

Factor VII. A small, inconsistent effect across studies was found among the 10 studies of a general population, the 3 studies of populations with CVD, and the 4 studies of people with dyslipidemia. The only statistically significant effects – both net increases in factor VII – were seen in 2 of the 3 studies of diabetic patients (one of which included only diabetics with dyslipidemia). The large increase in factor VII found by Hendra et al. in a 6 week study of fish oil versus olive oil supplements in non-insulin dependent diabetics was noted to be unexpected in light of a large decrease in Tg level [116].

Factor VIII. The single study of insulin dependent diabetics found a larger net increase of factor VIII than the studies of general populations, although the difference in this study was not significant. No study measured factor VIII in CVD or dyslipidemic populations.

von Willebrand Factor. With the exception of a low-dose arm in 1 study, the 6 studies of general populations found either net decreases or no effect in vWF, although none was statistically significant. The single study of a CVD population was the only study to find an

overall net increase in vWF level, although Leng et al. was also an anomaly in that the oil analyzed was primarily gamma-linolenic acid (GLA, 18:3 n-6), an omega-6 fatty acid, with a small amount of EPA [69].The only study to find a large, statistically significant decrease in vWF was 1 of the 2 studies of dyslipidemic patients. No study evaluated diabetic patients.

Covariates

Factor VII. Haines et al. found no association between change in factor VII with fish oil supplementation and either sex or Hgb A_{1c} in insulin dependent diabetics [115]. In contrast, in a study of non-insulin dependent diabetics, Dunstan et al. reported a significant positive association between the changes in factor VII and fasting blood sugar with a fatty fish diet; however, dietary fish significantly affected factor VII levels only in subjects who were not in a moderate exercise program [141]. Eritsland et al. reported no change in (lack of) effect of fish oil supplements in patients undergoing coronary bypass surgery after controlling for multiple factors including age, sex, weight, blood pressure, diabetes and CVD medications [142].

In possible contrast to the rest of the studies, only 1 of the 6 studies of male subjects, 3 of which were of younger men, found a net increase in factor VII; however all effects were small [46,89,138,140,147,149]. One study in which all subjects were on simvastatin [150] found a non-significant effect of fish oil supplements similar to other studies.

Factor VIII. Haines et al. found no relationship between effect of fish oil supplementation in insulin dependent diabetics who were taking aspirin on factor VIII and either sex or Hgb A_{1c} [115]. All other studies were in men, most of whom were under age 40 years. There were no other data relating to other covariates.

von Willebrand Factor. No study reported on correlations between effect on vWF and covariates. Notably, though, only 2 of the studies included women [69,150]. The effect of fish oil supplements in patients on simvastatin was similar to the effect of fish oil alone in other studies [150].

Dose and Source Effect

Factor VII. No study compared different doses of the same omega-3 fatty acid source. Across studies there does not appear to be a dose effect. Four studies compared different sources of omega-3 fatty acids. Hansen et al. (1993a) found no difference between fish oil triglycerides and fish oil ethyl esters [147]. Osterud et al. reported no difference in effect of different marine oils [74]. Freese et al. compared similar doses of fish oil and linseed oil supplements and found no difference between the 2 oils [143]. Agren et al. also did not report a difference in effect among fish oil supplementation, algae DHA oil supplementation, and fatty fish diet [140].

Factor VIII. Only Deslypere et al. compared different doses of fish oil supplements [85]. They reported no difference in effect of fish oil on factor VIII related to dose. None of the studies of fish oil supplements showed more than a marginal decrease in factor VIII level. In contrast, the single study of a flaxseed oil diet found a non-significant, approximately 6% net decrease in factor VIII activity and the single study of Mediterranean diet found a highly significant, approximately 7% net reduction in factor VIII activity. In the latter study, Mezzano et al. also found significant reductions in factor VII activity and fibrinogen levels, in contrast to most other studies [138]. They found no association between the effect on factor VIII and either ABO blood type (which is related to factor VIII level) or CRP, as a marker of inflammation.

von Willebrand Factor. Deslypere reported no difference in effect on vWF after 1 year in monks taking 3 different doses of fish oil supplements [85]. Hansen found similar effects among men taking either fish oil triglycerides or fish oil ethyl ester [147]. Across studies, though, the study by Seljeflot et al., which tested the largest dose of omega-3 fatty acid supplementation, found the largest, significant decrease in vWF. However, the study of mackerel paste diet, with a similar omega-3 fatty acid level, found no effect. The single study of plant oils found a non-significant decrease in vWF with an ALA-rich flaxseed oil diet that was similar to most marine oil studies.

Exposure Duration

Factor VII. Five studies measured factor VII levels at different time periods, ranging from 2 to 16 weeks [56,115,138,149,155]. No differences were seen in factor VII levels at any time point.

Factor VIII. Three studies measured factor VIII activity at different time periods. Haines et al. found no effect of fish oil supplements on factor VIII at either 3 or 6 weeks [115]. Deslypere et al. did find an occasional significant decrease of factor VIII from the second trial month on in multiple measurements done between 4 weeks and 12 months [85]. However, this effect was also seen in the olive oil group and no net differences were found. Mezzano et al. found similar responses to Mediterranean diet at both 1 and 3 months [138].

von Willebrand Factor. Three studies measured vWF at different time periods. Muller et al. found no change in vWF in either study arm at both 3 and 6 weeks [149]. Both Deslypere et al. and Leng et al. found that vWF levels fluctuated at different time points ranging from 3 weeks to 1 year, but that there were no differences among arms [69,85].

Sustainment of Effect

Factor VII. Only Freese et al. reported data on factor VII levels after stopping treatment [143]. There was no difference 4 weeks after finishing 4 weeks of treatment compared to either pre- or post-treatment levels.

Factor VIII and von Willebrand Factor. Only Deslypere et al. reported data on factor VIII activity and vWF after stopping treatment [85]. There was a large increase in factor VIII activity in all study arms, including the olive oil group, at both 1 and 2 months after stopping treatment. There were no differences between fish oil supplement and control groups. There was no difference in vWF after treatment.

Platelet Aggregation

(Table 3.20)

Platelet aggregation plays a central role in the pathogenesis of acute atherothrombosis and has been associated with cardiovascular disease in some, but not all, epidemiological studies. However, pharmacological agents that inhibit platelet aggregation, such as aspirin, clearly reduce the incidence of adverse clinical cardiovascular events. The most common method of measuring platelet aggregation involves *in vitro* tests of blood samples. Aggregating agents such as adenosine diphosphate (ADP) and collagen are added to the blood samples, or spontaneously occurring aggregation is measured. The resulting platelet aggregation is used as a measurement of the potential for platelets to aggregate in the human body. There is little agreement as to which

method is most meaningful and little standardization of dose of aggregating agent or test methodology. Omega-3 fatty acids may directly affect platelets, thus both reducing CVD but also possibly increasing bleeding risk.

We found 84 studies that met eligibility criteria and reported data on the effect of omega-3 fatty acids on platelet aggregation (See Table 3.1). Of these, we analyzed the 11 randomized trials with data on at least 15 subjects in parallel trials and 10 subjects in crossover trials who consumed omega-3 fatty acids and that also reported platelet aggregation in tabular or text format. Studies that presented platelet aggregation data graphically only were not analyzed. This additional criterion was used because of the particular difficulty in estimating data from graphs for this outcome and because of the large number of specific outcomes reported in each study.

Overall Effect [54,57,108,115,116,128,140,157-160]

Within the 11 studies, heterogeneous effects of omega-3 fatty acids were generally found depending on the aggregating agent, the dose of agent, and the measurement metric used. However, in most studies either no effect on platelet aggregation was found with omega-3 fatty acids or no difference in effect was seen between treatments and controls.

Sub-populations

Seven studies were performed in generally healthy individuals. Salonen et al., Junker et al., and Wensing et al. all found no effect of omega-3 fatty acid consumption and no difference with control groups in healthy men, non-obese individuals and elderly individuals, respectively [56,159,160]. Freese et al. (1994) found no significant effect from rapeseed oil supplements in male students; however, they did find an apparent comparative effect since Trisun sunflower oil, which was used as the comparison, significantly increased platelet aggregation [54]. Hansen et al., Freese et al. (1997a), and Agren et al. found mixed effects in younger individuals (Agren at al. in male students), with significantly decreased platelet aggregation in some study arms with some specific tests [128,140,157].

Two studies evaluated hypercholesterolemic subjects, both of which found no effect of omega-3 fatty acids on measures of platelet aggregation. An additional 2 studies included diabetic patients. Haines et al. reported no effect among insulin-dependent diabetics, while Hendra et al. reported small, but significant increases in spontaneous platelet aggregation among type 2 diabetics [115,116]. However, in the latter study it was also reported, without supporting evidence, that epinephrine-induced aggregation was unaffected by either treatment or control. No studies specifically included patients with known or suspected CVD.

Table 3.20 Effects of omega-3 fatty acids on platelet aggregation in randomized trials (4 to 15 weeks)

Author, Year	Omega-3 Fatty Acid Arm [a]				Control		Results [b]				Quality [c]				
	N	Source		g/d	N	Source	Method, Unit	Base	Net Δ	*P*	Summary	Jadad	Allocation Conceal	Applicability [d]	
		DHA/EPA Oils													
Hansen, 1993b	14 [e] Women	Cod liver oil	ED	5.3	14 [e]	No oil	Collagen 0.5 µg/mL, %	47.0	+1.2	NS	C	1	Un	GEN	II
							Collagen 4 µg/mL, %	95.0	+1.4	NS					
							ADP 2.5 µmol/L, %	81.0	-4.3	NS					
	20 [e] Men				20 [e]		Collagen 0.5 µg/mL, %	56.0	-24.7	<.01					
							Collagen 4 µg/mL, %	95.0	-2.6	NS					
							ADP 2.5 µmol/L, %	76.0	-5.9	NS					
Haines, 1986	19	Fish oil	ED	4.6	22	Olive oil	Collagen 1 µg/mL, Unit	49.3	-3.1	NS	B	2	Ad	IDDM	II
							Collagen 10 µg/mL, Unit	59.1	+2.2	NS					
Sirtori, 1992	12 [e]	Fish oil	ED	4.5	12 [e]	No oil	Collagen AC_{50}, mg/L [f]	0.35	+0.05	NS	C	2	Un	DysLip	II
							Iloprost IC_{50}, nmol/L [g]	0.65	+0.07	NS					
Hendra, 1990	35	Fish oil	ED	3.0	32	Olive oil	Spontaneous 10 min, [h]	77.3	+3.2	.06	B	4	Un	DM II	I
							Spontaneous 20 min, [h]	70.3	+4.4	.02					
							Spontaneous 30 min, [h]	67.4	+4.7	.02					
							Spontaneous 60 min, [h]	62.9	+4.2	.02					
Salonen, 1987	20	Fish oil	ED	2.7	24	Olive oil	ADP 2.3-9.0 µmol/L Aggregation extent, mV	16.2	+3.3	NS	B	3	Un	GEN	II
							ADP 2.3-9.0 µmol/L Aggregation velocity, mV/sec	0.16	+0.05	NS					
		Plant Oils													
Kwon, 1991	16	Canola oil diet	T	8-9% [i]	14	Safflower oil diet	Collagen 1 mg/L Maximum aggregation, Ω	43.5	0	NS	C	1	Un	DysLip	II
							Collagen 2 mg/L Maximum aggregation, Ω	46.3	+1.5	NS					
Junker, 2001	18	Rapeseed oil diet	T	2.5% [j]	40	Olive or Sunflower oil diet	ADP 0.5 µmol/L, %	7.8	+19.7 [k]	NS	C	1	Un	GEN	I
							ADP 2 µmol/L, %	27.1	+31.4 [k]	NS					
							Adrenaline 1 µmol/L, %	82.3	+9.9 [k]	NS					
							Adrenaline 4 µmol/L, %	85.1	7.8 [k]	NS					
							Spontaneous	7.2	+1.3 [k]	NS					
Freese, 1994	20	Rapeseed oil diet	A	2.3% [j]	20	Trisun Sunflower oil diet [L]	ADP 1 µmol/L slope, %/min	19.9	-5.4 [m]	.004	C	1	Un	GEN	III
							ADP 2 µmol/L slope, %/min	43.4	-9.5 [m]	.002					
							ADP 3 µmol/L slope, %/min	56.4	-6.6 [m]	.001					
							Thrombin 0.12 NIH/mL slope, %/min [n]	20.7	-1.0	NS					
							Thrombin 0.15 NIH/mL slope, %/min [n]	33.5	-3.8	.03					
							Thrombin 0.18 NIH/mL slope, %/min [n]	36.7	-3.0 [m]	.02					

Continued

Table 3.20 Effects of omega-3 fatty acids on platelet aggregation in randomized trials (continued)

Author, Year	Omega-3 Fatty Acid Arm [a]				Control		Results [b]				Quality [c]				
	N	Source		g/d	N	Source	Method, Unit	Base	Net Δ	*P*	Summary	Jadad	Allocation Conceal	Applicability [d]	
		Combinations													
Freese, 1997a	16	Fish oil	ED	5.2	--	--	ADP 1 µmol/L, %/min	34.4	+3.8 [o]	nd [p]	C	3	Un	GEN	II
							ADP 2 µmol/L, %/min	60.0	+4.6 [o]	nd [p]					
							ADP 3 µmol/L, %/min	72.3	-0.7 [o]	nd [p]					
							Collagen 0.5 µg/mL, %/min	53.3	-22.2 [o]	nd [p]					
							Collagen 1 µg/mL, %/min	81.2	-2.2 [o]	nd [p]					
							Collagen 3 µg/mL, %/min	99.6	-3.4 [o]	nd [p]					
	14	Linseed oil	A	5.9			ADP 1 µmol/L, %/min	34.8	-0.7 [o]	nd [p]					
							ADP 2 µmol/L, %/min	56.3	-1.6 [o]	nd [p]					
							ADP 3 µmol/L, %/min	68.8	-5.0 [o]	nd [p]					
							Collagen 0.5 µg/mL, %/min	44.8	-9.6 [o]	nd [p]					
							Collagen 1 µg/mL, %/min	78.6	-1.8 [o]	nd [p]					
							Collagen 3 µg/mL, %/min	94.3	+4.1 [o]	nd [p]					
Agren, 1997	14	Fish oil	ED	2.3	14	No oil	ADP 2 µmol/L, %T [n]	49.9	-5.8	NS	B	3	Un	GEN	III
							ADP 5 µmol/L, %T [n]	74.2	-9.3	NS					
							Collagen 50 µg/mL, %T [n]	51.3	-31.2	<.05					
	14	Algae DHA oil	D	1.7			ADP 2 µmol/L, %T [n]	37.2	+7.5	NS					
							ADP 5 µmol/L, %T [n]	64.5	-0.1	NS					
							Collagen 50 µg/mL, %T [n]	39.3	+13.7	NS					
	13	Fatty Fish diet	ED	1.1			ADP 2 µmol/L, %T [n]	35.1	+4.6	NS					
							ADP 5 µmol/L, %T [n]	70.0	-2.9	NS					
							Collagen 50 µg/mL, %T [n]	66.1	-20.7	<.05					
Wensing, 1999	14	Fish oil shortening	ED	1.6	11	Sunflower oil	ADP 1.5 µmol/L V_a, % [q]	48.2	+6.7	NS	B	2	Un	GEN	II
							ADP 1.5 µmol/L I_{max}, % [r]	69.6	+2.2	NS					
							Collagen 1.0 µg/mL V_a, % [q]	46.5	-6.2	NS					
							Collagen 1.0 µg/mL I_{max}, % [r]	65.7	+2.8	NS					
	13	Linseed oil shortening	A	6.5			ADP 1.5 µmol/L V_a, % [q]	52.9	-1.9	NS					
							ADP 1.5 µmol/L I_{max}, % [r]	73.3	-15.6	NS					
							Collagen 1.0 µg/mL V_a, % [q]	40.2	-3.8	NS					
							Collagen 1.0 µg/mL I_{max}, % [r]	50.2	+10.4	NS					

nd = no data

a A = ALA; D = DHA; E = EPA; ED = EPA+DHA; T = Total omega-3 fatty acids.

b Base = baseline level in treatment arm; Net Δ = net difference in change in omega-3 fatty acids arm compared with the change in control arm, see Methods; *P* = *P* value of difference between treatment and control arms; NS = not statistically significant.

c A = good quality; B = fair quality; C = poor quality; Jadad = Jadad Score (0-5, based on randomization, double blinding, and dropouts); Ad = adequate allocation concealment; In = inadequate allocation concealment; Un = allocation concealment unclear. See Methods.

d CVD = history of cardiovascular disease; DM I/II = diabetes mellitus type 1 or 2; DysLip = dyslipidemia; GEN = general, healthy population; N/IDDM = (non-) insulin dependent diabetes mellitus. I = broadly applicable; II = applicable to sub-group; III = narrow applicability. See Methods.

e Cross-over study.

f Concentration of collagen giving a 50% decrease in optical density.

g Concentration of Iloprost resulting in 50% inhibition of platelet aggregation.

h Percent platelets remaining after aggregation.

i Percent of total methyl esters in diet.

j Kcal.

k Difference compared to average change in 2 control groups.

L High linoleic acid (18:2 n-6) oil.

m No significant effect compared to baseline. Significant increase compared to Trisun oil, which increased platelet aggregation rate.

n No definition of unit provided.

o Pre-post difference (not compared to control).

p Not significant between treatments.

q Aggregation velocity.

r Maximal velocity.

Covariates

Hansen et al., recognizing that male and female sex hormones have different effects on platelet function, made an *a priori* evaluation of the potentially different effect of cod liver oil supplementation on platelet aggregation in men and women [157]. Healthy, young, normolipemic men and women were included in the study. A large, significant decrease in platelet aggregation with low dose collagen was seen in men on cod liver oil supplements, but not in women ($P < .01$ men vs. women). Otherwise the effect of fish oil was generally mixed and not different between the sexes. No explanation was offered for why the effect would have been seen only with low-dose collagen aggregation. In contrast, Haines et al. made the blanket statement that the baseline variables smoking, alcohol consumption, and sex were not related to the response to fish oil supplementation [115]. Four other studies included only men [54,57,140,159]. No clear difference was seen between these studies and studies that included both men and women. No other covariate was specifically analyzed in any study.

Dose and Source Effect

No study compared different doses of the same type of oil. Among the studies of fish oil supplements or diets, there was no clear association across studies between dose and change in platelet aggregation.

No significant effect was seen in any of the studies of plant oil supplements or diets, regardless of dose. Two studies compared fish oil (EPA+DHA) to linseed oil (ALA). Freese et al (1997a) was inconclusive regarding a difference between fish oil and linseed oil supplements [128]. However, Wensing et al. reported that platelet aggregation was prolonged by greater amounts in subject who consumed fish oil shortening compared to those who consumed linseed oil shortening [160]. Agren et al. compared 3 sources of EPA and/or DHA [140]. Collagen aggregation

was reduced in subjects on both fish oil supplementation and fish diet, but not in those consuming pure DHA oil. From this, they concluded that while omega-3 fatty acids impair platelet aggregation, DHA is less potent than fish oil or dietary fish at moderate doses.

Exposure Duration

Three studies measured platelet aggregation at different time points. Haines et al. and Junker et al. reported data at 3 and 6 weeks, and 2 and 4 weeks, respectively, but did not comment on a potential time effect [56,115]. However, no apparent difference in effect was seen between the earlier and later times. Kwon et al. noted that with 2 mg/L collagen aggregation a significant decrease in platelet aggregation was found at 3 weeks on canola oil diet, which reverted to baseline by 8 weeks [57].

Sustainment of Effect

Freese et al. (1997a) reported that the decrease in collagen-induced aggregation in the fish oil supplement arm did not return to baseline during a 12 week follow-up period, although, the other tests did [128].

Coronary Artery Restenosis

(Table 3.21, Figure 3.3)

The benefit of treatments given after percutaneous transluminal coronary angioplasty (PTCA) is often measured, in research studies, by performing a subsequent angiography and measuring the change in the luminal diameter at the sites of dilatation performed in the original angioplasty. The most common metric is restenosis rate, although there is no single standard definition of restenosis. Most researchers use minor variations of a 50% narrowing of the dilated vessel from the immediately post-dilation diameter. In theory, this level of restenosis corresponds with recurrence of angina, although clearly some patients develop symptoms with lesser levels of stenosis and some patients stay asymptomatic with greater levels of stenosis. If omega-3 fatty acids are effective at reducing clinical coronary artery disease, including angina and myocardial infarction, then the effect should be manifested in the diagnostic testing by angiography.

We found 17 studies that met eligibility criteria and reported data on coronary arteriography in patients taking omega-3 fatty acids (See Table 3.1). Of these, we analyzed the 12 randomized trials with data on restenosis rate after PTCA. Most studies re-evaluated patients at 6 months after PTCA. Maresta et al. started patients on omega-3 fatty acids 1 month prior to the initial PTCA [81]. In general, other studies started omega-3 fatty acid treatment up to a week prior to PTCA.

Overall Effect [63,64,81,161-169]

All studies compared a single dosage of fish oil supplementation to control. Definitions of restenosis, however, were not uniform as noted in the footnotes of the summary table. In particular, 3 studies included abnormal exercise tolerance tests (ETT) as a potential definition of

restenosis [166,167,169]. The results of random effects model meta-analysis are presented in both the Table 3.21 and Figure 3.3. Overall, although there is heterogeneity among the studies, there is a trend toward a net reduction of coronary artery restenosis with fish oil supplementation. The meta-analysis estimate is a lowering of risk of 14% (95% confidence interval –29%, +3%).

Table 3.21 Effects of omega-3 fatty acids on restenosis in randomized trials (approximately 3 months to 1 year)

Author, Year	Omega-3 Fatty Acid Arm [a]				Control		Results [b]			Quality [c]			Applicability [d]
	N	Source		g/d	N	Source	CR (%)	RR [e]	(95% CI)	Summary	Jadad	Allocation Conceal	
	DHA/EPA Oils												
Reis, 1989 [f]	124	Fish oil	T	6.0	63	Olive oil	22	1.60	(0.95, 2.68)	B	2	Un	CVD I
Cairns, 1996	312	Fish oil	ED	5.4	313	Corn oil	45	1.04	(0.88, 1.23)	B	3	Un	CVD II
Dehmer, 1988	43	Fish oil	ED	5.4	39	No oil	46	0.40	(0.20, 0.82)	B	3	Un	CVD II
Johansen, 1999	196	Fish oil	ED	5.0	192	Corn oil	45	1.03	(0.82, 1.28)	A	3	Ad	CVD I
Milner, 1989 [g]	84	Fish oil	ED	4.5	99	No oil	35	0.54	(0.32, 0.90)	B	3	Un	CVD I
Bairati, 1992a	59	Fish oil	ED	4.5	60	Olive oil	48	0.63	(0.40, 1.01)	B	5	Un	CVD I
Nye, 1990	35	Fish oil	ED	3.6	34	Olive oil	30	0.38 [h]	(0.17, 0.84)	C	4	Un	CVD I
Franzen, 1993	92	Fish oil	ED	3.1	83	Olive oil	35	0.93	(0.62, 1.41)	B	5	Ad	CVD II
Grigg, 1989	52	Fish oil	ED	3.0	56	Olive/corn	31	1.09 [i]	(0.65, 1.84)	C	3	Ad	CVD I
Bellamy, 1992	60	Fish oil	ED	3.0	53	No oil	40	0.80 [j]	(0.49, 1.32)	C	3	Un	CVD I
Kaul, 1992 [k]	58	Fish oil	ED	3.0	49	No oil	27	1.23	(0.68, 2.24)	B	2	Un	CVD II
Maresta, 2002	125	Fish oil	ED	2.6 [L]	132	Olive oil	41	0.76	(0.55, 1.06)	B	3	Un	CVD I
REM MA [m]	1,240				1,173			0.86	(0.71, 1.03)				

a A = ALA; D = DHA; E = EPA; ED = EPA+DHA; T = Total omega-3 fatty acids.
b CR = control rate (the rate of restenosis in the control arm); RR = relative risk; 95% CI = 95% confidence interval.
c A = good quality; B = fair quality; C = poor quality; Jadad = Jadad Score (0-5, based on randomization, double blinding, and dropouts); Ad = adequate allocation concealment; In = inadequate allocation concealment; Un = allocation concealment unclear. See Methods.
d Applicability based on generalizability to patients undergoing percutaneous transluminal coronary angioplasty (PTCA) for coronary stenosis. I = broadly applicable; II = applicable to sub-group; III = narrow applicability. See Methods.
e Relative risk calculated based on reported data.
f Three patients refused angiography and underwent exercise tolerance test instead. Angiographic restenosis defined as >70% narrowing.
g In asymptomatic patients, restenosis defined by abnormal exercise tolerance test. In patients with symptoms, restenosis defined by either exercise tolerance tests, angiography, or both.
h Based on lesions, not subjects
i Numbers in various sections of text and graph are not consistent. Data here derived from graph. Apparently, these numbers are based on numbers of lesions, but this is unclear.
j Only percentage of patients with restenosis reported. Percentage does not exactly match number of patients reported to have had follow-up restenosis.
k In asymptomatic patients, lack of restenosis defined by normal exercise tolerance test. Patients with symptoms or abnormal exercise tolerance tests underwent angiography.
L 5.1 g for 1 month before and 1 month after PTCA, then reduced to 2.6 g for an additional 5 months.
m Random effects model meta-analysis. See Methods.

Sub-populations and Covariates

Most studies included all patients who were undergoing first PTCA, therefore with known or suspected coronary artery disease. No study restricted eligibility to patients with either diabetes or dyslipidemia. A number of studies performed multivariate analysis including diabetic, lipid, and cardiovascular variables, generally finding no association between these covariates and

restenosis in the randomized trials. Only Bairati et al. commented about the effect of multivariate analysis on the relative risk of restenosis from fish oil supplement treatment [161]. The authors reported that after controlling for history of hypertension, myocardial infarction, and diabetes, and for smoking, body mass index, angina class, degree of stenosis, location and number of stenoses, and ejection fraction, the inverse association between fish oil supplementation and restenosis was stronger and of higher statistical significance (because of a higher risk profile in the fish oil group).

Reis et al. and Kaul et al. both compared relative risk of restenosis in men and women; neither found a significant difference in effect, although both found a higher (worse) relative risk in women than in men [166,169]. In men, the relative risks of restenosis were 1.33 and 1.29, respectively, compared to 2.20 and 1.78 in women. Notably, though, these 2 studies had the lowest control rates (the rate of restenosis in the control arm, a commonly used metric to estimate the underlying severity of disease) and were the only 2 studies with relative risks substantially greater than 1.0. Interestingly, the 1 study which was restricted to men, Dehmer et al., had about the lowest relative risk of restenosis among the studies.

Dose and Source Effect

No study compared doses of fish oils and all evaluated only fish oil. Across studies, no effect is apparent based on dose of fish oil supplement.

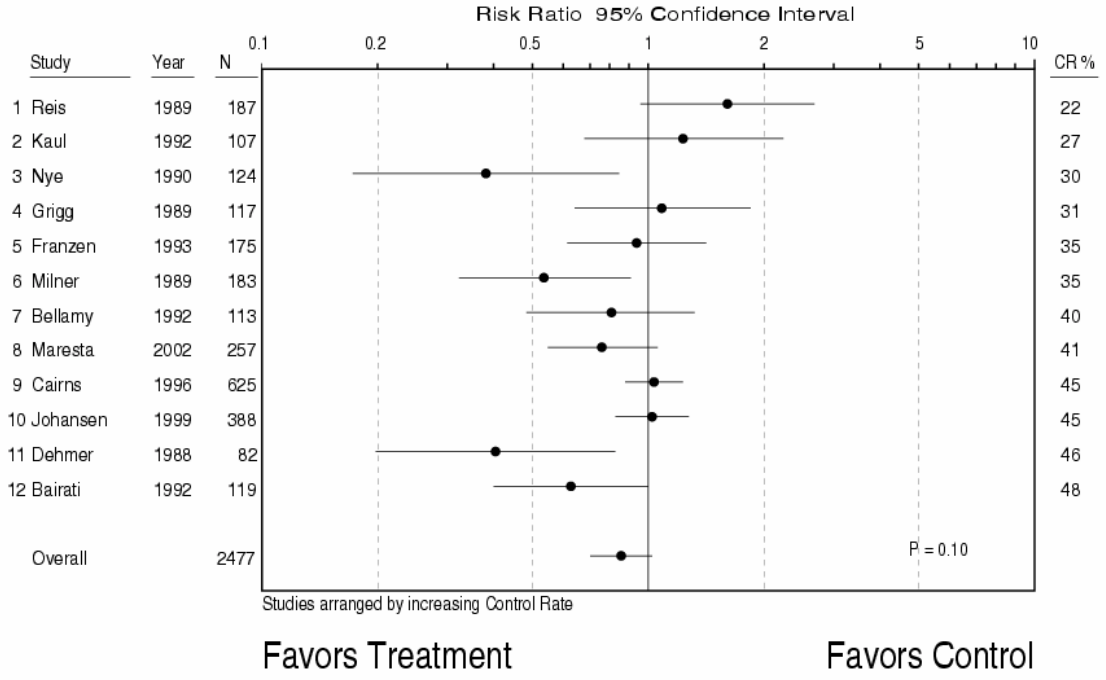
Exposure Duration

Each study evaluated restenosis at one time point only. Across studies, the duration of treatment does not appear to correlate with the relative risk of restenosis. In fact, both the longest study [168] (12 months) and the shortest study [163] (approximately 3-4 months) had similarly, low and statistically significant relative risks of restenosis.

Sustainment of Effect

No study re-evaluated for restenosis after stopping treatment.

Figure 3.3 Random effects model of effect of fish oil on coronary artery restenosis following percutaneous transluminal coronary angioplasty.



N = number of patients, except for 2 studies that reported number of lesions: Nye [168] had 35 patients on fish oil, 34 on control; Grigg [164] had 52 patients on fish oil, 56 on control. CR% = control rate, the restenosis rate in the control arm.

Carotid Intima-Media Thickness

(Table 3.22)

Ultrasound measurement of the thickness of the carotid arterial wall, termed carotid intima media thickness (IMT), has emerged as a practical technique that carries significant prognostic information in terms of future cardiovascular outcomes [170,171]. There are numerous methods of measuring carotid IMT, including using different sites and averaging different numbers of measurements. The more commonly reported methods include measurements of the common carotid artery and an average of multiple sites in the common and internal carotid arteries and the carotid bifurcation.

Four studies met eligibility criteria and reported data on the effect of omega-3 fatty acids on carotid IMT. Only one was a randomized trial of fish oil supplements. A second study reported IMT measurements only from the intervention arm of a randomized trial of ALA margarine. Two cross-sectional studies compared residents of a Japanese fishing village to a farming village and quartiles of white Americans based on ALA intake.

Table 3.22 Effects of omega-3 fatty acids on carotid intima-media thickness (mm) in studies (2 yr or cross-sectional)

Author, Year	Omega-3 Fatty Acid Arm [a] / Control Arm				Results [b]				Quality [c]				Applicability [d]
	N	Source		g/d	Arteries [e]	Base	Net Δ	*P*	Summary	Jadad	Allocation Conceal		
RCT													
		DHA/EPA Oils											
Angerer, 2002	87	Fish oil	ED	1.7	Overall; mean maximum	1.26	+0.02	NS	B	4	Ad	CVD	II
					CCA; mean maximum	0.86	+0.02	NS					
	84	Fatty acid			CB; mean maximum	1.54	+0.03	NS					
					ICA; mean maximum	1.11	+0.02	NS					
Longitudinal Cohort (No Control)					**Pre-Post Δ**								
		Plant Oils											
Bemelmans, 2002	95	ALA margarine	A	1.7	Overall; mean	0.83	+0.05 [f]	<.01 [g]	--	--	--	CVD	I
Cross-Sectional					**Cohort Δ**								
		Plant Oils											
Djousse, 2003 [h]	175	Mean total linolenic acid intake [i]	A	1.2	CCA; mean [j]	0.64	-0.06	.01 Trend	--	--	--	GEN	I
	176		A	0.8		0.60	-0.10						
	174		A	0.6		0.63	-0.07						
	173		A	0.4		0.70	--						
	175	Mean total linolenic acid intake [i]	A	1.2	CB; mean [j]	0.94	-0.05	.0008 Trend					
	176		A	0.8		0.86	-0.13						
	174		A	0.6		0.91	-0.08						
	173		A	0.4		0.99	--						
	175	Mean total linolenic acid intake [i]	A	1.2	ICA; mean [j]	0.71	-0.01	NS Trend					
	176		A	0.8		0.70	-0.02						
	174		A	0.6		0.70	-0.02						
	173		A	0.4		0.72	--						
		Fish and Mediterranean Diets											
Yamada, 1997	248	Fishing village	F	146	CCA; mean	0.70	-0.03	<.05	--	--	--	GEN	II
	197	Farming village	F	84		0.73	--						

a A = ALA; D = DHA; E = EPA; ED = EPA+DHA; F = Fish; T = Total omega-3 fatty acids.

b Base = baseline level in treatment arm; Net Δ = net difference in change in omega-3 fatty acids arm compared with the change in control arm, see Methods; Pre-Post Δ = change in omega-3 fatty acid arm (no control); Cohort Δ = difference in IMT between cohort and reference cohort (cross-sectional); *P* = *P* value of difference between treatment and control arms; NS = not statistically significant.
c A = good quality; B = fair quality; C = poor quality; Jadad = Jadad Score (0-5, based on randomization, double blinding, and dropouts); Ad = adequate allocation concealment; In = inadequate allocation concealment; Un = allocation concealment unclear. See Methods.
d CVD = history of cardiovascular disease; DM I/II = diabetes mellitus type 1 or 2; DysLip = dyslipidemia; GEN = general, healthy population; N/IDDM = (non-) insulin dependent diabetes mellitus. I = broadly applicable; II = applicable to sub-group; III = narrow applicability. See Methods.
e CB, Carotid bifurcation; CCA, Common carotid artery; ICA, Internal carotid artery.
f Change from baseline.
g Compared to baseline.
h The N's represent the number of subjects with baseline data. The numbers of subjects with each measurement of arteries' IMTs were not recorded. The range of number of arteries measured is 181-348 across arteries.
i By staff-administered semi-quantitative food-frequency questionnaire.
j Mean values adjusted for sex, age, energy, waist-to-hip ratio, field center, and smoking status.

Overall Effect [51,79,172,173]

The only placebo-controlled randomized trial found small, non-significant net thickening of carotid IMT, using 4 different measurements at 24 months, with fish oil supplementation. The uncontrolled cohort of subjects consuming ALA margarine had a significant thickening in IMT at 2 years. However, the absolute change in IMT in this cohort of subjects was similar to the absolute change in IMT in the fish oil supplementation arm in the randomized trial (an absolute increase of between 0.05 mm and 0.11 mm in the study by Angerer et al.) [79,172]. The cross-sectional studies both found that people with greater dietary intake of omega-3 fatty acids, either as total linolenic acid or as fish, had significantly thinner IMTs than those with less intake.

Sub-populations and Covariates

Other than study design, the primary difference between the studies that found no effect and the studies that found a beneficial effect of omega-3 fatty acids is that the former were both trials in patients with cardiovascular disease and the latter were both studies of generally healthy individuals. There is insufficient data, however, to conclude that the differences were due to study populations. There is no evidence among people with diabetes or hyperlipidemia. Bemelmans et al. performed a regression analysis of predictors of change in IMT among subjects taking ALA margarine [172]. Age, sex, blood pressure, LDL, and weight were not predictive of change in IMT. In addition, change in intake of polyunsaturated fatty acids, cholesterol and alcohol were not predictive of change in IMT. Change in intake of saturated fatty acids (SFA) was positively associated, and change in intake of fruit was negatively associated, with change in IMT in univariate analysis but not in multivariate analysis (although it is not clear what factors were included in multivariate analysis since none was significant).

In the cross-sectional study, IMT was greater in older than younger subjects in both the fishing and farming villages. Among younger villagers, IMT was non-significantly lower in the fishing village than the farming village; however, in subjects in their seventh and eighth decades IMT was marginally greater in the fishing village.

Dose and Source Effect, Exposure Duration, Sustainment of Effect

There are insufficient data to draw conclusions regarding dose effect, oil type, duration of intervention or exposure, or sustainment of effect after stopping omega-3 fatty acids.

Exercise Tolerance Test

(Table 3.23)

The exercise tolerance test (ETT), or stress test, measures the heart's aerobic exercise capacity and is a common test to determine clinical severity of coronary artery disease. The standard method of performing ETT is with the modified Bruce protocol on a treadmill. Some studies instead used a bicycle ergometer. A wide range of different metrics are used to measure patients' performance.

All eligible studies that reported data on the effect of omega-3 fatty acids on ETT were included; 6 studies qualified. Three were randomized trials and 3 were longitudinal cohort studies without control arms of subjects with known coronary artery disease who were treated with fish oil supplements.

Table 3.23 Effects of omega-3 fatty acids on treadmill and bicycle exercise tolerance tests in studies (6 weeks-6 months)

Author, Year	Omega-3 Fatty Acid Arm [a] / Control Arm				Results [b]				Quality [c]			Applicability [d]	
	Source		g/d	N	Test	Base	Net Δ	*P*	Summary	Jadad	Allocation Conceal		
	DHA/EPA Oils												
RCTs													
Solomon, 1990	Fish oil	ED	4.6	5	Work load producing angina, kwatt-sec	18.87	-1.47	NS	B	4	Un	CVD	II
	Olive oil			5									
Franzen, 1993	Fish oil	ED	3.1	92	Exercise capacity, kwatt-sec	29	+5.2	NS	B	5	Ad	CVD	II
	Olive oil			83	Sum ST depression, mV	2.0	-0.2	nd					
Salachas, 1994	Fish oil	ED	3.0	20	Exercise duration, min	8.2	+1.7	<.05	B	4	Un	CVD	II
	Olive oil			19	Maximum double product [e]	16.5	+6.2	<.05					
Longitudinal Cohorts (No Control)						**Pre-Post Δ**							
Warren, 1988	Cod liver oil	E	3.1	7	Peak exercise RPP [f]	18,800	+300	NS	--	--	--	CVD	II
				7	Ratio resting/exercise RPP [f]	0.45	-0.08	<.05					
				6	Time to ischemia, min	7.6	+0.9	NS					
Verheugt, 1986	Fish oil	ED	3.0	5	Exercise duration, min	6.8	-0.2	NS	--	--	--	CVD	I
					Max ST depression, mm	2.6	+0.2	NS					
Toth, 1995	Fish oil	T	1.7	10	Peak exercise TPR [g]	730 [h]	-40 [h]	<.01	--	--	--	CVD DysLip	II
					Peak exercise Cardiac Index [i]	6.3 [h]	+1.0 [h]	<.05					
					Relative aerobic capacity, %	70 [h]	+10 [h]	<.01					
					ST score	1.2 [h]	-0.4 [h]	<.05					

nd = no data

a A = ALA; D = DHA; E = EPA; ED = EPA+DHA; F = Fish; T = Total omega-3 fatty acids.

b Base = baseline level in treatment arm; Net Δ = net difference in change in omega-3 fatty acids arm compared with the change in control arm, see Methods; Pre-Post Δ = change in omega-3 fatty acid arm (no control); *P* = *P* value of difference; NS = not statistically significant.
c A = good quality; B = fair quality; C = poor quality; Jadad = Jadad Score (0-5, based on randomization, double blinding, and dropouts); Ad = adequate allocation concealment; In = inadequate allocation concealment; Un = allocation concealment unclear. See Methods.
d CVD = history of cardiovascular disease; DM I/II = diabetes mellitus type 1 or 2; DysLip = dyslipidemia; GEN = general, healthy population; N/IDDM = (non-) insulin dependent diabetes mellitus. I = broadly applicable; II = applicable to sub-group; III = narrow applicability. See Methods.
e Maximum heart rate x maximum systolic pressure; likely divided by 1000
f Rate-pressure product; equivalent to work load.
g Total peripheral resistance during exercise, measured using impedance-cardiography.
h Estimated from graph.
i Cardiac index during exercise, measured using impedance-cardiography.

Overall Effect [64,174-178]

The 3 randomized trials each found a small relative improvement in exercise capacity in subjects with coronary artery disease who took fish oil supplements compared to those who took olive oil supplements. However, with a single exception, exercise capacity measurements improved in all study arms, regardless of whether subjects consumed fish oil or olive oil supplements. The maximum double product (heart rate multiplied by blood pressure) fell by a non-significant amount in the olive oil arm in Salachas et al. [174].

Warren et al. evaluated 7 patients with stable angina who took cod liver oil supplements for 6 weeks [178]. Exercise workload and time to ischemia improved, although the changes were not significant. The ratio of resting to exercise workload fell significantly. Verheugt et al. studied 5 men with moderate to severe exercise-induced angina [177]. They were given fish oil for 6 months. The patients' angina was sufficiently severe that all ETTs both before and after treatment were discontinued because of angina symptoms. Essentially no change was found in either exercise duration or maximal ST depression. Toth et al. enrolled 10 men with coronary artery disease and hyperlipidemia [176]. They fish oil supplements for 2 months. A variety of measures of cardiac function significantly improved.

Overall, given the small number of studies and subjects, the different metrics used across studies, and the lack of placebo control in half the studies, only limited conclusions can be drawn about the effect of omega-3 fatty acids in improving cardiac function in patients with coronary artery disease. The studies suggest that fish oil consumption may benefit exercise capacity among patients with coronary artery disease, although the effect may be small.

Sub-populations, Dose Effect, Duration, Sustainment of Effect

There is no evidence regarding different doses, duration of fish oil consumption, other omega-3 fatty acids, the effect in various sub-populations, or sustainment of effect.

Heart Rate Variability

(Table 3.24)

Heart rate variability is measured on 24-hour ambulatory electrocardiography recordings. A number of different measurements can be used to estimate heart rate variability. The studies of omega-3 fatty acids primarily measured the mean standard deviation (SD) of the RR interval (the time between heart beats). Abnormal QRS complexes were excluded. The larger the SD of the RR interval (SDNN), the greater the variability of the time between heart beats. An increase in SDNN is protective against ventricular arrhythmias and, in post-myocardial infarction patients, is protective against mortality [179,180]. Notably, both beta blockers and angiotensin converting enzyme inhibitors both increase heart rate variability [179].

Only one set of investigators, in Denmark, have reported data on the effect of omega-3 fatty acids on heart rate variability in studies that met eligibility criteria. They analyzed 2 sets of subjects in randomized trials and also analyzed the cross-sectional data of one of the sets of subjects.

Table 3.24 Effects of omega-3 fatty acids on heart rate variability – SD of RR (msec) – in studies (12 weeks or cross-sectional) [a]

Author, Year	Omega-3 Fatty Acid Arm [b]				Control		Results [c]			Quality [d]				Applicability [e]
	N	Source		g/d	N	Source	Base	Net Δ	*P*	Summary	Jadad	Allocation Conceal		
RCTs														
		DHA/EPA Oils												
Christensen, 1999	20	Fish oil	ED	5.9	20	Olive oil	136	+13	NS	B	4	Un	GEN	II
	20	Fish oil	ED	1.7			164	+3	NS					
Christensen, 1996	26	Fish oil	ED	4.3	23	Olive oil	115	+18	<.05	B	4	Ad	CVD	III
Cross-sectional				**Frequency**				**Cohort Δ**						
		Fish and Mediterranean Diets												
Christensen, 1997 [f]	18	Fish diet		≥2x/wk	9	No fish	119	+16 [g]	NS	--	--	--	CVD	III
	25	Fish diet		1x/wk			122	+19 [g]	NS					

a Standard deviation of RR intervals on 24 hour ambulatory electrocardiography recordings.
b A = ALA; D = DHA; E = EPA; ED = EPA+DHA; T = Total omega-3 fatty acids.
c Base = baseline level in treatment arm; Net Δ = net difference in change in omega-3 fatty acids arm compared with the change in control arm, see Methods; Cohort Δ = difference in IMT between cohort and reference cohort (cross-sectional); *P* = *P* value of difference between treatment and control arms; NS = not statistically significant.
d A = good quality; B = fair quality; C = poor quality; Jadad = Jadad Score (0-5, based on randomization, double blinding, and dropouts); Ad = adequate allocation concealment; In = inadequate allocation concealment; Un = allocation concealment unclear. See Methods.
e CVD = history of cardiovascular disease; DM I/II = diabetes mellitus type 1 or 2; DysLip = dyslipidemia; GEN = general, healthy population; N/IDDM = (non-) insulin dependent diabetes mellitus. I = broadly applicable; II = applicable to sub-group; III = narrow applicability. See Methods.
f Cross-sectional evaluation of baseline data from Christensen, 1996.
g Difference between fish cohort and no-fish cohort.

Overall Effect [181-183]

One randomized controlled trial was performed in 60 healthy volunteers who took either low or high dose fish oil supplements, or olive oil capsules for 12 weeks [183]. No significant effect was

found either within study arms or compared to olive oil. The authors concluded that among all subjects, fish oil supplementation had no effect on heart rate variability.

In a randomized trial of 49 patients who had had a recent myocardial infarction and had a ventricular ejection fraction below 0.40 those who consumed fish oil supplements (for 12 weeks) had a significant increase in SDNN compared to controls [181]. The authors concluded that omega-3 fatty acids may increase heart rate variability in survivors of myocardial infarction which may be protective against ventricular arrhythmias and mortality.

The same patients with recent myocardial infarction were divided at baseline into 3 groups based on their regular level of fish consumption [182]. Both groups who consumed at least 1 fish meal per week had greater SDNN than those who did not consume fish, though the difference was not statistically significant. This finding may suggest that dietary fish consumption increases SDNN and thus is protective against ventricular arrhythmia.

Sub-populations and Covariates

Neither study directly compared healthy subjects with those with CVD. Neither examined subjects with either diabetes or dyslipidemia. While the effect of fish oil supplementation appeared greater in the study of subjects with recent myocardial infarction, there is insufficient evidence to compare the effect in subjects with or without heart disease.

In the study of healthy subjects, sub-group analyses based on sex and baseline SDNN suggested that the effect of fish oil supplementation was greatest in the 18 men with below median (<150 msec) baseline SDNN. However, data were not reported for the other 3 subgroups (women and those with above median SDNN).

Dose and Source Effect and Exposure Duration

The study among healthy subjects compared low and high dose fish oil supplementation. While it appears that there may be a trend toward increasing SDNN with higher dose fish oil, it is noteworthy that the subjects on high dose fish oil had no change in their SDNN while those on olive oil had a decrease in SDNN. Both trials lasted 12 weeks. There is no evidence regarding the effect of duration of intervention or exposure.

Sustainment of Effect

Neither study re-examined subjects after stopping fish oil supplementation.

Tissue Levels of Dietary Omega-3 Fatty Acids

(Tables 3.25-3.31, Figures 3.4-3.6 [Figures at end of Tissue Levels section])

As noted in Chapter 1, in theory, the most immediate outcome related to omega-3 fatty acid intake is a change in tissue levels of the fatty acids. In this section, we review studies that examined the correlation between omega-3 fatty acid intake and tissue levels. Among studies analyzed for other outcomes, we found 60 studies that reported data on the association between omega-3 fatty acid consumption and changes in omega-3 fatty acid composition in various tissues. Of these, we analyzed the 33 largest randomized trials that reported percent phospholipid

levels in either plasma or serum or in 1 of 4 blood cell membranes (Table 3.25). For plasma and serum phospholipid composition and for platelet phospholipid composition we analyzed randomized trials with data on at least 25 subjects and crossover trials with at least 20 subjects in omega-3 treatment arms. Because few studies reported erythrocyte, granulocyte, or monocyte membrane phospholipid compositions, we analyzed all eligible randomized trials.

Summary (Table 3.26)

Meta-regression revealed direct relationships between dose of consumed EPA+DHA and changes in measured levels of EPA and DHA, either as plasma or serum phospholipids, platelet phospholipids, or erythrocyte membranes. The correlation between dose and change in level appears to be fairly uniform, where 1 g supplementation of EPA and/or DHA is associated with, approximately, a 1% increase in EPA+DHA level. Granulocyte and monocyte membrane phospholipid levels also increased by roughly similar amounts after omega-3 fatty acid supplementation in individual studies. In these studies, ALA level did not change significantly after supplementation in any blood marker. In most studies, there was a decrease in arachidonic acid (AA, 20:4 n-6) level, which corresponded to the increase in EPA+DHA level.

Among eligible studies, only 3 included ALA supplementation arms [53,143,160]. The dose of ALA in these 3 studies ranged from 4.5 to 9.5 g/d. The studies consistently found an increase in both ALA and EPA levels in the blood markers, at these doses of ALA. In contrast, there was no significant change in DHA level when lower dose of ALA was used (up to 6.8 g/d) but in the study arm that received 9.5 g/d ALA a significant increase in DHA level was also found.

Table 3.25. Studies reporting plasma/serum, platelet, erythrocyte, and other phospholipid changes

Study	Study Design	N [a]	Plasma or Serum PL	Platelet PL	RBC PL	Granulocyte PL	Monocyte PL
Agren, 1988	RCT	29		√	√		
Agren, 1991	RCT	49		√	√		
Agren, 1996	RCT	41	√				
Angerer, 2002	RCT	87			√		
Bonaa, 1992	RCT	72	√				
Brox, 2001	RCT	80	√				
Cobiac, 1991	RCT	25	√				
Dehmer, 1988	RCT	43		√			
Dunstan, 1997	RCT	26		√ [b]			
Dunstan, 1999	RCT	26			√ [b]		
Finnegan, 2003	RCT	116	√ [c]				
Freese, 1997b	RCT	29		√ [c]			
Green, 1990	Crossover	27	√	√	√		
Grimsgaard, 1997	RCT	147	√				
Grundt, 1995	RCT	28	√				
Haines, 1986	RCT	19			√		
Hansen, 1989	Crossover	40	√				√
Hansen, 1993b	Crossover	34	√				
Hendra, 1990	RCT	37		√			
Leigh-Firbank, 2002	Crossover	55		√			
Luo, 1998	Crossover	10			√		
Madsen, 2003	RCT	40		√		√	
McVeigh, 1993	Crossover	23		√			
Mori, 1994	RCT	85		√			
Mori, 1999	RCT	27	√ [b]				
Mori, 2000	RCT	36	√	√			

Nenseter, 2000	RCT	34	√	
Osterud, 1995	RCT	106	√	
Rivellese, 1996	RCT	8		√
Sacks, 1994	RCT	60	√	
Solomon, 1990	RCT	5		√
Wensing, 1999	RCT	27		√ [c]
Woodman, 2002	RCT	35	√	

PL = phospholipids; RBC = red blood cell (erythrocyte); RCT = randomized controlled trial.
a Subjects consuming omega-3 fatty acids.
b Study reported total omega-3 fatty acids only. Not in the meta-regression analyses.
c Study included an ALA treatment arm.

Table 3.26. Association of EPA+DHA consumption and tissue levels. Meta-Regression Results

Markers	Studies	Arms [a]	Slope	SE [b] of Slope	Intercept	r^2	*P* value
Plasma or serum phospholipids	15	28	0.93	0.20	1.41	0.45	<.001
Excluding studies with incomplete data [c]	12	24	1.24	0.20	0.89	0.63	<.001
Platelet phospholipids	12	20	0.74	0.16	1.16	0.52	<.001
Excluding studies with incomplete data [d]	10	18	0.80	0.12	1.25	0.72	<.001
Erythrocyte membrane	10	13	0.63	0.40	3.22	0.11	.14
Excluding studies with incomplete data [e]	9	12	1.05	0.37	2.69	0.39	.02
Granulocyte membrane	1	2	--				
Monocyte membrane	1	1	--				

a Number of separate study arms of subjects who consumed omega-3 fatty acids.
b Standard error. Use number of treatment arms to back-calculate standard deviation.
c Hansen, 1989 [146]; Hansen, 1993b [157]; Green, 1990 [101]; Sacks, 1994 [75] were excluded because only change of EPA in the marker's phospholipid profile was reported.
d Green, 1990 [101]; Hendra, 1990 [116] were excluded because only change of EPA in the marker's phospholipid profile was reported.
e Green, 1990 [101] was excluded because only the change of EPA in the marker's phospholipid profile was reported.

Plasma or Serum Phospholipid Composition [48,53,62,66,74,90,97,100,101,120,129,131,132,146,157,184] (Table 3.27, Figure 3.4)

EPA/DHA. For plasma and serum phospholipid composition, 16 randomized trials with 30 omega-3 fatty acid arms were initially included; however, we excluded 1 study that reported only total omega-3 fatty acid dose and levels [131]. Among the 15 trials of EPA and/or DHA supplementation (which had 28 treatment arms), the dose of EPA+DHA ranged from 0.2 to 5.8 g/day. Study populations include general healthy population, and people with diabetes, dyslipidemia or cardiovascular diseases. Meta-regression shows a significant dose-response relationship between the dietary EPA and DHA supplementations and the changes in EPA+DHA compositions in plasma or serum phospholipids across studies. Across studies, the effect was similar regardless of source of EPA or DHA. Three studies compared purified EPA to purified DHA [66,120,132]. All found that purified EPA increased EPA and decreased DHA in plasma phospholipid and that purified DHA increased DHA by about 4 to 7 times as much as EPA in plasma phospholipid; however, combined EPA+DHA was increased by about the same amount by both fatty acids.

Meta-regression equation ($r^2 = 0.45$, $P < .001$):

Change in Plasma/Serum EPA+DHA Level (%) = 0.93 x [EPA+DHA Intake (g/day)] + 1.41

Because 4 studies reported only EPA levels, we re-analyzed the data with only the 12 studies with a complete EPA and DHA profile of plasma/serum phospholipids. As expected, since no study excluded DHA levels, the revised meta-regression equation indicates that the EPA+DHA level increases by a greater amount for each unit of omega-3 fatty acid supplementation and the r^2 was greater than in the meta-regression that included all studies.

Meta-regression equation ($r^2 = 0.63$, $P < .001$):

Change in Plasma/Serum EPA+DHA Level (%) = 1.24 x [EPA+DHA Intake (g/day)] + 0.89

ALA. One study also evaluated 2 linseed/rapeseed oil supplementation doses, which included primarily ALA with minimal EPA and DHA [53]. Finnegan et al. found that with higher dose ALA (9.5 g/d), EPA, DHA and ALA levels all significantly increased. With lower dose ALA (4.5 g/d), EPA and ALA levels rose by a degree consistent with the lower dose of omega-fatty acids; although DHA levels did not change. In the remaining study arms of fish oils and sunflower oils, small amounts of ALA (<= 1.5 g/d) did not affect ALA levels. In this study, a daily dose of 9.5 g or 4.5 g ALA (with 0.3 g EPA+DHA) had similar effects on plasma EPA levels as a daily dose of 1.7 g or 0.8 g EPA+DHA (with 1.4 g ALA), respectively. The plasma level of AA did not decrease in either ALA arm.

Table 3.27 Effect of omega-3 fatty acid supplementation on fatty acid profile of serum/plasma phospholipids in randomized trials (6 weeks to 14 months)

Study, Year	Omega-3 Fatty Acid Arms [a]				Base ED (%) [b]	Results (Δ%) [c]				Quality [d]				
	Control Arm													
	N	Source		g/d		AA [f]	ALA	EPA	DHA	Summary	Jadad	Allocation Conceal	Applicability [e]	
		EPA/DHA Oils												
Bonaa, 1992	72	Fish oil	ED	5.1	11.8	-1.00	0.00	+5.10	+1.70	B	4	Un	DysLip	I
	74	Corn oil	ED	0	11.5	+0.60	+0.10	-0.20	-0.20					
Green, 1990	27 [g]	Fish oil	ED	4.3	nd	0.00		+2.60		B	4	Un	DysLip	II
		Corn/Olive oil	ED	0	nd	nd		nd						
Grimsgaard, 1997	75	EPA ester	E	4.0	6.0	-0.98	-0.05	+4.65	-0.55	A	5	Un	GEN	I
	72	DHA ester	D	4.0	6.0	-0.82	-0.02	+0.47	+3.30					
	77	Corn oil	ED	0	6.2	+0.11	+0.01	-0.06	-0.10					
Mori, 2000	19	Purified EPA	E	4.0	nd	-3.00		+8.25	-0.25	B	4	Un	DysLip	II
	17	Purified DHA	D	4.0	nd	-2.25		+1.00	+7.25					
	20	Olive oil	ED	0	nd	+0.10		-0.10	+0.50					
Woodman, 2002	17	Purified EPA	E	4.0	5.9			+8.64	-1.29	B	3	Un	DM II	II
	18	Purified DHA	D	4.0	6.0			+1.09	+6.71					
	16	Olive oil	ED	0	6.8			nd	nd					
Grundt, 1995	28	Fish oil	ED	3.4	nd	-0.60		+3.80	+1.50	B	2	Un	DysLip	II
	28	Corn oil	ED	0	nd	-0.30		-0.50	-0.40					
Brox, 2001	40	Cod liver oil	ED	3.3	5.2	-0.34	-0.39	+1.27	+1.49	C	1	Un	DysLip	I
	40	Seal oil	ED	2.6	4.8	+0.14	-0.35	+2.61	+1.81					
	36	No oil	ED	0	5.1	+0.26	+0.91	+0.04	+0.81					
Osterud, 1995 [h]	26	Cod liver oil	ED A	3.1 0.2	nd	-0.25	-0.09	+2.36	+1.51	C	2	Un	GEN	I
	27	Seal/Cod liver oil	ED A	2.8 0.2	nd	-0.30	-0.04	+2.66	+1.85					
	27	Seal oil	ED A	2.4 0.2	nd	-0.20	-0.07	+2.01	+1.29					

Study, Year	Omega-3 Fatty Acid Arms [a] Control Arm N	Source	g/d	Base ED (%) [b]	Results (Δ%) [c] AA [f]	ALA	EPA	DHA	Quality [d] Summary	Jadad	Allocation Conceal	Applicability [e]	
	26	Whale oil	ED 1.7 A 0.2	nd	-0.15	-0.06	+1.14	+0.74					
	28	No oil	ED 0	nd	nd	nd	nd	nd					
Hansen, 1989	40 [g]	Cod liver oil	ED 5.8	nd	-0.70		+4.10		C	1	Un	GEN	I
		No oil	ED 0	nd	+0.10		0.00						
Hansen, 1993b	34 [g]	Cod liver oil	ED 5.3	nd			+5.44		B	1	Un	GEN	II
		No oil	ED 0	nd			nd						
Sacks, 1994	60	Fish oil	ED 2.4	nd			+2.95		C	3	Un	CVD	I
	60	Olive oil	ED 0	nd			+0.10						
Nenseter, 2000	34	Fish powder	ED 0.2	5.4	+0.20	-0.10	+0.20	+0.20	B	3	Un	GEN	II
	36	Cellulose	ED 0	6.1	+0.30	+0.10	-0.30	0.00					
		Fish Diets											
Mori, 1999	13	Fish & WMD [i]	T 3.7	nd	Total n-3 fatty acids: +6.0				B	2	Un	GEN	II
	16	WMD [i]	T nd	nd	Total n-3 fatty acids: -1.5								
	14	Fish & ERD [j]	T 3.7	nd	Total n-3 fatty acids: +5.0								
	16	ERD [j]	T nd	nd	Total n-3 fatty acids: -1.0								
		Combinations											
Cobiac, 1991	13	Fish oil	ED 4.6	2.5	-0.10	0.00	+5.80	+3.10	B	2	Un	GEN	II
	12	Fish [k]	ED 4.5	2.5	-0.70	+0.20	+3.10	+3.50					
	6	No oil	ED 0	2.4	+0.60	+0.10	-0.20	-0.20					
Agren, 1996	14	Fish oil	ED 2.3	nd			+4.00	+2.20	B	3	Un	GEN	III
	14	Algae DHA oil	D 1.7	nd			+0.50	+3.10					
	13	Fish [k]	ED 1.1	nd			+1.50	+1.50					
	14	No oil	ED 0	nd			-0.10	0.00					
Finnegan, 2003	28	Fish oil margarine and Fish oil	ED 1.7 A 1.4	5.02	-0.56	-0.07	+1.13	+2.71	A	4	Un	DysLip	I
	30	Fish oil margarine	ED 0.8 A 1.3	4.41	+0.44	-0.08	+0.80	+1.61					
	29	Rapeseed/Linseed margarine	ED 0.3 A 9.5	4.38	+0.53	+0.46	+1.22	+0.21					
	29	Rapeseed/Linseed margarine	ED 0.3 A 4.5	5.34	+0.74	+0.15	+0.95	-0.13					
	30	Sunflower seed oil margarine	ED 0.5 A 1.5	5.47	+0.64	-0.05	+0.28	-0.08					

nd = no data; n-3 = omega-3;

a A = ALA; D = DHA; E = EPA; ED = EPA+DHA; T = total omega-3 fatty acids.

b Baseline EPA + DHA profile (% of total fatty acids) of plasma/serum phospholipids.

c Δ% = Difference of the marker's profile (post-treatment minus pre-treatment).

d A = good quality; B = fair quality; C = poor quality; Jadad = Jadad Score (0-5, based on randomization, double blinding, and dropouts); Ad = adequate allocation concealment; In = inadequate allocation concealment; Un = allocation concealment unclear. See Methods.

e CVD = history of cardiovascular disease; DM I/II = diabetes mellitus type 1 or 2; DysLip = dyslipidemia; GEN = general, healthy population; N/IDDM = (non-) insulin dependent diabetes mellitus. I = broadly applicable; II = applicable to sub-group; III = narrow applicability. See Methods.

f Arachidonic acid (20:4 n-6)

g Cross-over study.

h Differences are from the control after 10-week treatment. Assumed control's profile didn't change from baseline, so differences from the controls would be approximately equal to the Δ%.

i Weight-maintaining diet.

j Energy-restricted diet.

k Chemically analyzed.

Platelet Phospholipid Composition [68,71,95,96,101,116,122,123,132,137,143,163]

(Table 3.28, Figure 3.5)

EPA/DHA. For platelet phospholipid composition, we analyzed 12 randomized trials with 21 omega-3 fatty acid arms. All of these studies evaluated EPA and/or DHA supplementation. One treatment arm was ALA; therefore, there were 20 EPA and/or DHA treatment arms. The dose of EPA+DHA ranged from 0.8 to 5.9 g/day. Study populations include general healthy population and people with diabetes, dyslipidemia, or cardiovascular diseases. Meta-regression results show a significant dose-response relationship between the dietary EPA and DHA supplementations and the changes in EPA+DHA compositions in platelet phospholipids across studies. Studies that used fish or fish combined with fish oil supplement treatments generally had greater increases in platelet phospholipid EPA+DHA amounts than studies of fish oil supplements. This effect was seen in Mori, et al. (1994), which compared fish, fish oil supplements, and combination fish and fish oil [71]. They reported that the largest increase in DHA occurred in the groups consuming fish. In contrast to the finding in plasma phospholipids, Mori et al. (2000) reported that platelet EPA+DHA levels rose more in subjects taking DHA than in subjects taking EPA, although it is not reported whether this difference is statistically significant [132].

Meta-regression equation ($r^2 = 0.52$, $P < .001$):

Change in Platelet EPA+DHA Level (%) = 0.74 x [EPA+DHA Intake (g/day)] + 1.16

As was the case for plasma/serum phospholipid levels, the re-analysis of the platelet phospholipid data that excluded the 2 studies without a complete EPA and DHA profile indicates a larger increase in EPA+DHA level and a larger r^2 than in the complete meta-regression.

Meta-regression equation ($r^2 = 0.72$, $P < .001$):

Change in Platelet EPA+DHA Level (%) = 0.80 x [EPA+DHA Intake (g/day)] + 1.25

ALA. One study also evaluated linseed oil supplementation, which included only ALA without EPA or DHA [143]. Freese et al. found that a 5.9 g/d ALA supplementation significantly increased EPA and ALA platelet phospholipid levels. However, the effect on EPA levels was small in comparison to the effect of a similar dose of fish oil (+0.41% vs. +3.32% for 5.2 g/d EPA+DHA). In addition, DHA levels were unaffected. The AA level decreased in the ALA arm.

Table 3.28 Effect of omega-3 fatty acid supplementation on fatty acid profile of platelet phospholipids in randomized trials (6 weeks to 4 months)

Study, Year	Omega-3 Fatty Acid Arms [a]				Base ED (%) [b]	Results (Δ%) [c]				Quality [d]				
	Control Arm													
	N	Source		g/d		AA [f]	ALA	EPA	DHA	Summary	Jadad	Allocation Conceal	Applicability [e]	
		EPA/DHA Oils												
Madsen, 2003	20	Fish oil	ED	5.9	3.6	-4.49	-0.03	+3.82	+0.92	B	3	Un	GEN	I
	20	Fish oil	ED	1.7	3.2	-1.97	+0.01	+1.27	+0.36					
	20	Olive oil	ED	0	3.2	+0.19	+0.01	+0.01	-0.06					
Dehmer, 1988	43	Fish oil	ED	5.4	0.6	-2.50		+3.66	+2.30	B	3	Un	CVD	III
	39	No oil	ED	0	nd	nd		nd	nd					
Green, 1990	27 [g]	Fish oil	ED	4.3	nd	-0.50		+1.90		B	4	Un	DysLip	II
		Corn/Olive oil	ED	0	nd	nd		nd						
Mori, 2000	19	Purified EPA	E	4.0	nd	-4.80		+3.80	-0.60	B	4	Un	DysLip	II
	17	Purified DHA	D	4.0	nd	-2.40		+0.60	+4.20					
	20	Olive oil	ED	0	nd	-0.60		+0.05	+0.10					
Leigh-Firbank, 2002	55 [g]	Fish oil	ED	3.0	3.3	+2.90		+2.60	+1.11	B	3	Un	DysLip	I
		Olive oil	ED	0	3.3	+0.60		+0.20	+0.10					
McVeigh, 1993	23 [g]	Fish oil	ED	3.0	2.5	-3.00		+1.70	+2.70	A	4	Un	DM II	II
		Olive oil	ED	0	2.5	-0.40		-0.10	+0.30					
Hendra, 1990	37	Fish oil	ED	3.0	nd			+1.75		B	4	Un	DM II	I
	37	Olive oil	ED	0	nd			-0.02						
		Fish Diets												
Dunstan, 1997	26	Fish and exercise	T	3.6	nd	Total n-6: -5.80		EPA+DPA+DHA: +4.80		B	2	Un	NIDDM DysLip	I
	23	No fish and exercise	T	nd	nd	nd		nd						
Agren, 1988	14	Fish	ED	0.8	4.5	-2.1	+0.10	+1.20	+1.20	B	3	Un	GEN	III
	15	Fish and low SFA [h]	ED	0.8	4.4	-2.6	+0.10	+0.80	+1.30					
	19	Control diet	ED	0.05	nd	nd	nd	nd	nd					
Agren, 1991	22	Fish	ED	0.8	3.8	-1.30		+0.70	+0.70	B	2	Un	GEN	III
	23	Control diet	ED	0.1	3.7	-0.10		0.00	0.00					
	27	Fish and exercise	ED	0.8	3.6	-0.90		+0.70	+0.70					
	27	Control diet and exercise	ED	0.1	3.8	+0.10		0.00	0.00					
		Combinations												
Freese, 1997b	14	Fish oil	ED A	5.2 0.1	5.2 [i]	-3.35	-0.21	+3.32 [i]	+0.88	C	3	Un	GEN	II
	15	Linseed oil	ED A	0 5.9	4.7 [i]	-0.79	+0.39	+0.41 [i]	-0.14					
Mori, 1994	16	Fish [j] and Fish oil (40% [k])	ED	5.2	5.2	-5.00		+3.75	+2.50	B	2	Un	GEN	II
	17	Fish oil (40% [k])	ED	4.2	5.2	-4.75		+3.25	+1.50					
	17	Fish [j] (40% [k])	ED	3.0	5.2	-3.75		+2.50	+2.50					
	17	Fish oil (40% [k])	ED	2.1	5.2	-1.50		+1.60	+0.50					
	18	Control [L] oils (40% [k])	ED	nd	5.2	+0.75		-0.05	-0.40					
	18	Fish [j] (40% [k])	ED	3.0	5.2	-4.00		+2.30	+2.50					
	17	Control [L] oils (40% [k])	ED	nd	5.2	-0.40		-0.20	-0.50					

nd = no data; n-6 = omega-6
a A = ALA; D = DHA; E = EPA; ED = EPA+DHA; T = total omega-3 fatty acids.
b Baseline EPA + DHA profile (% of total fatty acids) of platelet phospholipids.
c Δ% = Difference of the marker's profile (post-treatment minus pre-treatment).
d A = good quality; B = fair quality; C = poor quality; Jadad = Jadad Score (0-5, based on randomization, double blinding, and dropouts); Ad = adequate allocation concealment; In = inadequate allocation concealment; Un = allocation concealment unclear. See Methods.
e CVD = history of cardiovascular disease; DM I/II = diabetes mellitus type 1 or 2; DysLip = dyslipidemia; GEN = general, healthy population; N/IDDM = (non-) insulin dependent diabetes mellitus. I = broadly applicable; II = applicable to sub-group; III = narrow applicability. See Methods.
f Arachidonic acid (20:4 n-6)
g Crossover study.
h Low saturated fatty acid diet.
i Plus some 22:0.
j Chemically analyzed.
k Percent of fat in diet
L Olive/Palm/ Safflower oils

Erythrocyte Membrane Phospholipid Composition [79,88,95,96,101,115,134,141,160,175] (Table 3.29, Figure 3.6)

EPA/DHA. For erythrocyte membrane phospholipid composition, 10 randomized trials with 15 omega-3 fatty acid arms were included. All of these studies evaluated EPA and/or DHA supplementation. One study included 2 ALA treatment arms; therefore, there were 13 EPA and/or DHA treatment arms. The dose of EPA+DHA ranged from 0.8 to 4.6 g/day. Study populations include general healthy population and people with diabetes, dyslipidemia or cardiovascular diseases. Meta-regression results show no significant dose-response relationship between the dietary EPA and DHA supplementations and the changes in EPA plus DHA compositions in platelet phospholipids. No clear difference is seen in effect based on source of omega-3 fatty acids. No study compared different sources of EPA+DHA oil.

Meta-regression equation ($r^2 = 0.11$, $P = .14$):
Change in Erythrocyte EPA+DHA Level (%) = 0.63 x [EPA+DHA Intake (g/day)] + 3.22

The re-analysis of the data, excluding 1 study by Green et al. who did not report the change in DHA levels, greatly affected slope and statistical significance of the meta-regression equation [101]. The large effect of this single study can be explained by outlier status of the study. The change in EPA level reported in this study is considerably lower than the change in EPA+DHA levels in studies with similar supplementation doses.

Meta-regression equation ($r^2 = 0.39$, $P < .02$):
Change in Erythrocyte EPA+DHA Level (%) = 1.05 x [EPA+DHA Intake (g/day)] + 2.69

ALA. One study also evaluated a diet enriched in ALA and that contained no EPA or DHA among both young (16-33 years old) and old (60-78 years old) subjects [160]. Wensing et al. found that a 6.8 g/d ALA supplementation significantly increased both EPA and ALA levels but not DHA level. The effects on the changes in EPA and ALA compositions were larger among older subjects than among younger subjects. The higher dose ALA (6.8 g/d) had a smaller effect on EPA levels (+0.20% and +0.40%, for younger and older subjects, respectively) than a lower dose

of EPA+DHA (1.6 g/d, +1.30%). The AA level decreased among old subjects while it increased among young subjects.

Table 3.29 Effect of omega-3 fatty acid supplementation on fatty acid profile of red blood cell (erythrocyte) membrane/ghosts in randomized trials (6 weeks to 2 years)

Study, Year	Omega-3 Fatty Acid Arms [a]				Base ED (%) [b]	Results (Δ%) [c]				Quality [d]				
	Control Arm													
	N	Source		g/d		AA [f]	ALA	EPA	DHA	Summary	Jadad	Allocation Conceal	Applicability [e]	
		EPA/DHA Oils												
Haines, 1986	19	Fish oil	ED	4.6	6.1	-2.20		+3.77	+2.23	B	2	Ad	IDDM	II
	22	Olive oil	ED	0	6.1	0.00		-0.05	-0.22					
Solomon, 1990	5	Fish oil	ED	4.6	6.7	-3.44		+5.92	+2.19	B	4	Un	CVD	II
	5	Olive oil	ED	0	6.4	+0.23		-0.07	-0.31					
Green, 1990	27 [g]	Fish oil	ED	4.3	nd	-2.00		+2.70		B	4	Un	DysLip	II
		Corn/Olive oil	ED	0	3.6	nd		nd						
Rivellese, 1996	8	Fish oil	ED	1.97	5.8	-2.30		+1.50	+1.60	A	3	Un	DysLip NIDDM	II
	8	Olive oil	ED	0.0	5.7	0.10		-0.10	-0.30					
Luo, 1998	10 [g]	Fish oil	ED	1.8	6.3			+1.44 [h]	+1.33 [h]	C	3	Un	DM II	II
		Sunflower oil	ED	0	6.3			nd	nd					
Angerer, 2002	87	Fish oil	ED	1.65	nd			+2.60	+4.20	B	4	Ad	CVD	II
	84	Fatty acid	ED	nd	nd			+0.10	+0.10					
		Fish Diets												
Dunstan, 1999	14	Fish and moderate exercise	T	3.6	nd	Total n-6: -4.50		EPA+DPA+DHA: +6.60		B	2	Un	NIDDM DysLip	I
	11	No fish and moderate exercise	T	nd	nd	Total n-6: +0.60		EPA+DPA+DHA: 0.00						
	12	Fish and light exercise	T	3.6	nd	Total n-6: -6.7		EPA+DPA+DHA: +8.40						
	12	No fish and light exercise	T	nd	nd	nd		nd						
Agren, 1988	14	Fish	ED	0.8	8.8	-2.70	0.00	+1.30	+3.20	B	3	Un	GEN	III
	15	Fish and low SFA [i]	ED	0.8	9.3	-2.40	0.00	+0.90	+2.70					
	19	Control diet	ED	0.05	nd	nd	nd	nd	nd					
Agren, 1991	22	Fish	ED	0.8	9.2	-1.30		+0.70	+1.80	B	2	Un	GEN	III
	23	Control diet	ED	0.1	8.7	+0.10		0.00	-0.10					
	27	Fish and exercise	ED	0.8	8.7	-1.20		+0.80	+2.00					
	27	Control diet and exercise	ED	0.1	8.6	+0.40		0.00	-0.10					
Wensing, 1999	14	EPA+DHA	ED	1.6	4.5	-0.80	0.00	+1.30	+0.80	B	2	Un	GEN	II
	13	ALA (old)	ED A	0.0 6.8	4.1	-0.70	+0.40	+0.40	-0.30					
	12	ALA (young)	ED A	0.0 6.8	4.0	+0.90	+0.20	+0.20	-0.10					
	11	Oleic acid	ED	0.0	3.8	-0.20	0.00	+0.00	+0.10					

nd = no data; n-6 = omega-6

a A = ALA; D = DHA; E = EPA; ED = EPA+DHA; T = total omega-3 fatty acids.
b Baseline EPA + DHA profile (% of total fatty acids) of erythrocyte phospholipids.
c Δ% = Difference of the marker's profile (post-treatment minus pre-treatment).
d A = good quality; B = fair quality; C = poor quality; Jadad = Jadad Score (0-5, based on randomization, double blinding, and dropouts); Ad = adequate allocation concealment; In = inadequate allocation concealment; Un = allocation concealment unclear. See Methods.
e CVD = history of cardiovascular disease; DM I/II = diabetes mellitus type 1 or 2; DysLip = dyslipidemia; GEN = general, healthy population; N/IDDM = (non-) insulin dependent diabetes mellitus. I = broadly applicable; II = applicable to sub-group; III = narrow applicability. See Methods.
f Arachidonic acid (20:4 n-6)
g Crossover study.
h Difference from the control after 2-month treatment. Assumed control's profile didn't change from baseline, so differences from the controls would be approximately equal to the Δ%.
i Low saturated fatty acid diet.

Granulocyte Membrane Phospholipid Composition [137] (Table 3.30)

One randomized controlled trial examined the changes of EPA+DHA composition in granulocyte membrane phospholipids after fish oil supplementation. Madsen et al. found that EPA and DHA compositions in granulocyte phospholipids significantly increased after 12 weeks of fish oil supplement treatment, while no significant changes were found in the placebo group [137]. In addition, the change in DHA profile was significantly larger in the higher-dose fish oil supplementation group than in the lower-dose fish oil group.

Table 3.30 Effect of omega-3 fatty acid supplementation on fatty acid profile of granulocyte membrane in randomized trials (12 weeks)

Study, Year	Omega-3 Fatty Acid Arms [a]				Base ED (%) [b]	Results (Δ%) [c]				Quality [d]				
	Control Arm													
	N	Source		g/d		AA [f]	ALA	EPA	DHA	Summary	Jadad	Allocation Conceal	Applicability [e]	
		EPA/DHA Oils												
Madsen, 2003	20	Fish oil	ED	5.9	2.2	-2.71	-0.03	+3.50	+0.57	B	3	Un	GEN	I
	20	Fish oil	ED	1.7	2.1	-1.21	0.00	+1.25	+0.29					
	20	Olive oil	ED	0	2.0	+0.03	+0.01	+0.04	-0.02					

a A = ALA; D = DHA; E = EPA; ED = EPA+DHA; T = total omega-3 fatty acids.
b Baseline EPA + DHA profile (% of total fatty acids) of granulocyte phospholipids.
c Δ% = Difference of the marker's profile (post-treatment minus pre-treatment).
d A = good quality; B = fair quality; C = poor quality; Jadad = Jadad Score (0-5, based on randomization, double blinding, and dropouts); Ad = adequate allocation concealment; In = inadequate allocation concealment; Un = allocation concealment unclear. See Methods.
e CVD = history of cardiovascular disease; DM I/II = diabetes mellitus type 1 or 2; DysLip = dyslipidemia; GEN = general, healthy population; N/IDDM = (non-) insulin dependent diabetes mellitus. I = broadly applicable; II = applicable to sub-group; III = narrow applicability. See Methods.
f Arachidonic acid (20:4 n-6).

Monocyte Membrane Phospholipid Composition [146] (Table 3.31)

One crossover study examined the changes of EPA+DHA composition in monocyte phospholipids after cod-liver oil supplementation. Hansen, et al. showed the EPA profile in monocyte phospholipids significantly increased, while the arachidonic acid profile significantly decreased after 8 weeks of cod liver oil supplement treatment compared to the no treatment controls [146].

Table 3.31 Effect of omega-3 fatty acid supplementation on fatty acid profile of monocyte phospholipids in randomized trials (8 weeks)

Study, Year	Omega-3 Fatty Acid Arms [a]				Base ED (%) [b]	Results (Δ%) [c]				Quality [d]				
	Control Arm													
	N	Source		g/d		AA [f]	ALA	EPA	DHA	Summary	Jadad	Allocation Conceal	Applicability [e]	
		EPA/DHA Oils												
Hansen, 1989	40 [g]	Cod liver oil	ED	5.8	nd	-4.00 [h]		+3.00 [h]		C	1	Un	GEN	I
		No oil	ED	0	nd	nd		nd						

nd = no data

a A = ALA; D = DHA; E = EPA; ED = EPA+DHA; T = total omega-3 fatty acids.

b Baseline EPA + DHA profile (% of total fatty acids) of monocyte phospholipids.

c Δ% = Difference of the marker's profile (post-treatment minus pre-treatment).

d A = good quality; B = fair quality; C = poor quality; Jadad = Jadad Score (0-5, based on randomization, double blinding, and dropouts); Ad = adequate allocation concealment; In = inadequate allocation concealment; Un = allocation concealment unclear. See Methods.

e CVD = history of cardiovascular disease; DM I/II = diabetes mellitus type 1 or 2; DysLip = dyslipidemia; GEN = general, healthy population; N/IDDM = (non-) insulin dependent diabetes mellitus. I = broadly applicable; II = applicable to sub-group; III = narrow applicability. See Methods.

f Arachidonic acid (20:4 n-6)

g Cross-over study.

h Difference from the control after 8-week treatment. Assumed control's profile didn't change from baseline, so differences from the controls would be approximately equal to the Δ%.

Figure 3.4 Association between EPA and/or DHA supplementation and changes in EPA+DHA composition in plasma or serum phospholipids (PL)

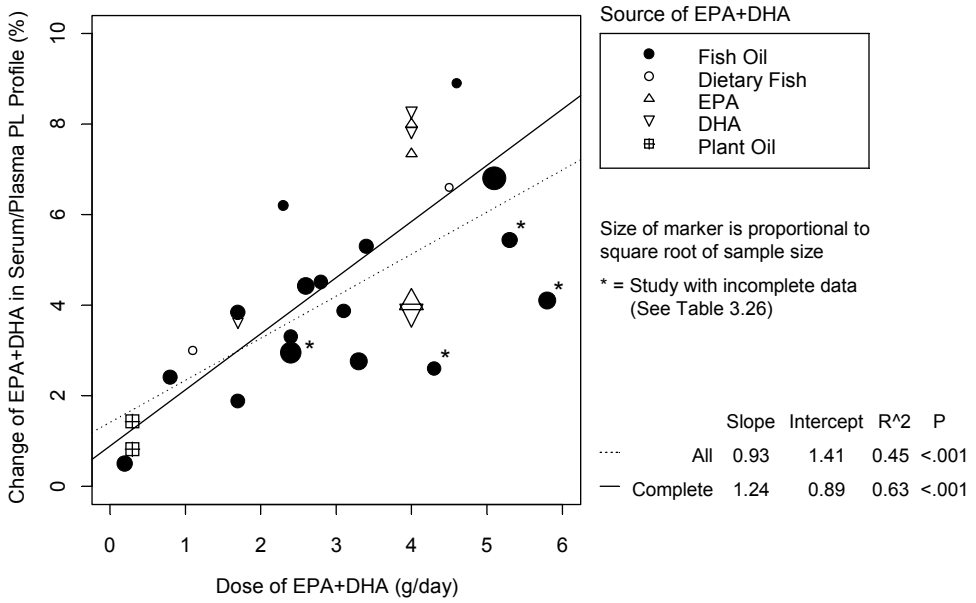


Figure 3.5 Association between EPA and/or DHA supplementation and changes in EPA+DHA composition in platelet phospholipids (PL)

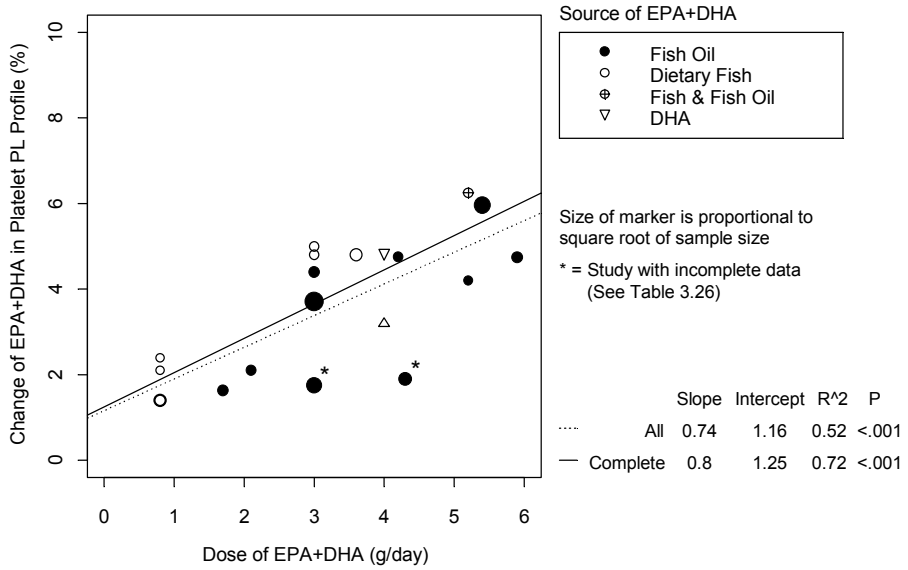
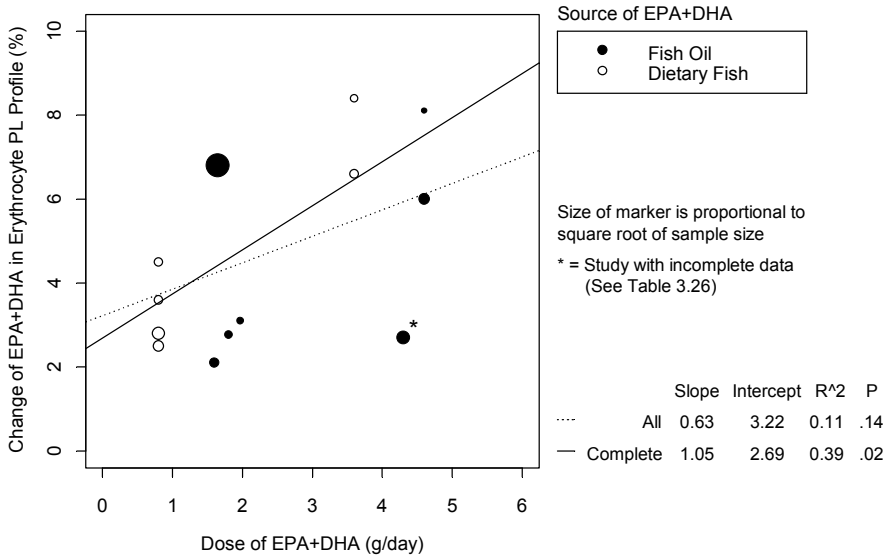



Figure 3.6 Association between EPA and/or DHA supplementation and changes in EPA+DHA composition in red blood cell (RBC, erythrocyte) membrane phospholipids (PL)